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Ovarian function after autotransplantation of the adenohypophysis in the pig

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OVARIAN FUNCTION AFTER AUTOTRANSPLANTATION OF THE ADENOHYPOPHYSIS IN THE PIG

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INTRODUCTION

Since the late 1920's when it was established that the pituitary gland is responsible for regulating the functions of the gonads, thyroid, adrenals, and mammary glands, as well as growth, research workers have attempted to elucidate the factors which influence and ultimately control the pituitary gland. Several *in vivo* methods for investigating the influence of the central nervous system have developed. These include pituitary transplantation, pituitary stalk transection, and induction of hypothalamic lesions. These techniques have demonstrated that the central nervous system controls the adenohypophysis in laboratory animals such as the rat, mouse, guinea pig, and hamster. Within the past 15 years some of these techniques have been applied to domestic animals such as the goat, sheep, cow, and pig.

It has been well documented that the pituitary gland is essential for maintenance of corpora lutea beyond the normal length of the estrous cycle in the pig. The presence of a gravid uterus or the absence of the nongravid uterus is essential for maintenance of corpora lutea beyond 16 days in this species. The porcine hypophysial luteotropin(s) has not been established. Likewise, little is known about how luteotropin(s) secretion is controlled by the central nervous system in the pig.

This research was conducted to investigate the influence of the adenohypophysis of the pig on gonadal function in the absence of control by the central nervous system. Corpora lutea were induced with desiccated porcine pituitary and human chorionic gonadotropin (HCG) in hypophysectomized and adenohypophysial autotransplanted, immature female pigs. The effect of the nongravid uterus on control of luteal function also was a part of this study. Hypophysectomized and adenohypophysial autotransplanted pigs were hysterectomized 8 to 10 days following induction of ovulation to remove the luteolytic influence of the uterus. Other pigs bearing adenohypophysial autotransplants were laparotomized, but the uterus remained in situ. Luteal function was evaluated later by histological examination and progesterone analysis of the tissue.

REVIEW OF LITERATURE

Early work involving pituitary transplantation techniques produced conflicting and often confusing results. Factors contributing to this situation were incomplete hypophysectomies, small numbers of experimental animals, lack of adequate control animals, and homotransplantations into intact animals. However, more recent work has also produced conflicting results due to some of these same factors, as well as a lack of knowledge concerning peripheral blood levels of hypothalamic releasing factors during various physiological states.

Some of the earliest work was confined to determining the ability of pituitary tissue to survive as an autotransplant or homotransplant in sites remote from the hypothalamus. The number of animals used and the resulting physiological data were often limited. By 1940, it was established that anterior pituitary tissue was capable of persisting for prolonged periods of time as viable auto- or homotransplants in several species. This was based mostly on histological evidence, but physiological data also appeared to support this finding.

One of the first published accounts of hypophysial transplantation was that of Crowe et al. (1909). Pituitary transplants were placed in the rectus muscle, bone marrow, and the cerebral cortex in totally and partially hypophysec-

tomized dogs. The lives of the pituitary transplanted dogs were prolonged beyond those observed in hypophysectomized controls. Most grafts became extensively invaded with connective tissue, therefore the observed effects were probably the result of a leaching of hormones from the pituitary graft. The best site for transplantation was the cerebral cortex. What appeared to be viable tissue was found at this site. Holweg and Junkmann (1932) implanted pituitaries into the kidneys of nonhypophysectomized rats which were later castrated. After one month the pituitary gland in the host showed characteristic changes due to castration; whereas castration cells were absent in the pituitary grafted tissue. Similar results concerning castration cells were reported by Martins (1936a). Anterior pituitary tissue was grafted into one adult male and four adult female rats 16 to 100 days after hypophysectomy. Animals were sacrificed 1 to 3 months after transplantation. With the exception of one female, which had four abnormally long estrous cycles (12 days), the ovaries showed abnormal follicular development. Castration cells were not found in pituitary transplants of castrated, hypophysectomized, pituitary-transplanted rats, as compared with those found in intact, castrated animals. Martins believed that the sex hormones must have acted via the central nervous system to influence the anterior pituitary. Similar results were

obtained by Westman and Jacobsohn (1940).

Haterius et al. (1935a, b) reported on homotransplantation of pituitary glands into the anterior chamber of the eye in nonhypophysectomized guinea pigs and rabbits. After 4 months the grafts appeared to undergo some hyperplasia, and occasional mitotic figures were seen. The existence of vascular connections could not be established, therefore, the authors concluded that these grafts obtained nutrients from the surrounding fluid of the eye chamber. The anterior chamber of the eye in rats was used as a transplantation site by Buxton (1936). The pituitary gland was autotransplanted in 35 rats, whereas seven hypophysectomized and nonhypophysectomized animals received homotransplants. Of these 42 rats seven possessed viable transplants 3 to 6 weeks later. The surviving transplants were well vascularized and consisted of adenohypophysial tissue; the neurohypophysis degenerated. In 1935 Gardner and Hill homotransplanted pituitaries into the testes of 22 to 50 day-old mice. Pituitary tissue was found in 11 of 13 mice 2 to 12 weeks after transplantation. The authors believed the distribution of acidophils, basophils, and chromophobes was similar to in situ anterior pituitary tissue. Mitoses of adenohypophysial cells were not observed. Cellular constituents and survival of multiple homotransplants in nonhypophysectomized mice of closely inbred

strains were described by Wolfe et al. in 1940. Pituitary glands were homotransplanted near the mammary glands of 20 animals. Viable grafts were found 7 to 10 months following transplantation. Loeb and Kirtz (1939) also found viable anterior pituitary grafts 8 to 10 months after transplantation in nonhypophysectomized mice.

Evidence for Gonadotropin Secretion by Ectopic Adenohypophysial Transplants

Males

After attempting several methods of replacement therapy in hypophysectomized rats, Smith (1930) and Smith and Engle (1927) reported a reversal of the hypophysectomy syndrome by daily implants of adenohypophysial tissue. In males the testes enlarged, spermatogenesis resumed, and sexual activity and fertility were restored. In females ovarian follicles developed and the reproductive tract repaired. When treatment stopped the reproductive organs atrophied again. Therefore these implants were probably not viable pituitary grafts.

In 1935 May reported that 2 months after intraocular transplantation of pituitary tissue in two hypophysectomized rats, their hair coat, body growth, and physical activity returned to those of intact animals. Testicular histology was normal and spermatozoa were present. Later work by May (1955) indicated the same gonad-stimulating capacity of

grafted anterior pituitary tissue in male mice. Mice weighing 8 to 12 grams were hypophysectomized. One month later the atrophied right testis contained spermatocytes, but no spermatozoa. A pituitary gland of a newborn mouse was then grafted under the tunica albuginea of one testis. One month later the recovered graft appeared to have grown and contained cells similar to in situ anterior pituitary. The seminiferous tubules of the remaining testis were enlarged and contained numerous spermatozoa. Gonadal stimulation by intratesticular pituitary homotransplants was reported by Hill and Gardner (1936) in only two male mice. The intratesticular grafts became well vascularized within 3 weeks. In a later study mice received pituitaries from female littermates at 23 and 42 days after hypophysectomy. Testicular histology revealed development of interstitial tissue with the production of spermatozoa by 4 months after transplantation. Unfortunately neither animal was completely hypophysectomized. Fragments of approximately 1.5% and 2.8% of the volume of a normal mouse pituitary were present at autopsy. However, the authors believed they were functionally complete hypophysectomies because previous work indicated that more than 5% of the anterior lobe must be present in the sella turcica to maintain gonadal function.

The ability of anterior pituitary tissue to survive as

a viable transplant after being cultured *in vitro* has been demonstrated. In 1936, Haymaker and Anderson cultivated rat adenohypophysial tissue *in vitro* before grafting it into 89 hypophysectomized male rats. They hoped this procedure would result in increased viability of the subsequent graft. Seventeen animals appeared to possess a functional graft 45 to 105 days after transplantation. Spermatogenesis was restored in three of these rats. The thyroids of three others contained areas in which the follicles were lined with cuboidal epithelium. Five animals with viable grafts had definite repair of the adrenal cortex and three others showed adrenal cortical changes suggestive of repair. Body weight loss was decreased in the grafted rats as compared with that of the hypophysectomized controls. Petrovic and Lavillaureix (1957) reported similar work with guinea pigs. After tissue culture of adenohypophysial fragments for 3 to 4 weeks these fragments were transplanted in testes of immature guinea pigs. Contrary to the work of Haymaker and Anderson (1936) spermatogenesis was not restored in the grafted animals, however there were indications of interstitial cell stimulation where the graft was in contact with the testicle. The cells of the grafts were described as degranulated. In 1966, Martinovitch *et al.* homotransplanted three or more anterior pituitaries from 3 week-old rats to either the anterior chamber of the eye or

beneath the kidney capsule in adult hypophysectomized female rats. These grafts were either previously cultured *in vitro* or transplanted immediately after removal from the donors. Ovarian growth with the development of graffian follicles resulted in 13 of 15 rats bearing pituitary grafts, regardless of whether these grafts were cultivated *in vitro* or not. There appeared to be a correlation between the number of pituitaries transplanted and the degree of stimulation of ovarian tissue in 14 of the 15 grafted rats. Another study by Martinovitch (1950) demonstrated the ability of pituitary tissue cultured *in vitro* to survive as a functional graft.

Other workers have investigated the secretory function of anterior pituitary transplants in male guinea pigs. Work by Schweizer *et al.* in 1940 demonstrated a secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) in hypophysectomized male guinea pigs bearing intraocular pituitary homotransplants. FSH secretion was indicated by the presence of all stages of spermatogenesis, sperm in the epididymides, and motile sperm in ejaculates. Less interstitial tissue was present in the testes compared with intact guinea pigs, but LH secretion was indicated by androgenic stimulation of the seminal vesicles. The same response was obtained whether the donor pituitary was from a male or female. Petrovic

and Aron, 1958 and Aron et al. 1953) used intratesticular pituitary homografts in adult, hypophysectomized guinea pigs. The homografts were made 2 to 20 weeks prior to hypophysectomy and the animals were autopsied 2 days to 16 weeks after hypophysectomy. Both testicles were maintained compared with hypophysectomized control animals, but the graft bearing testicle possessed greater hypertrophy of the interstitial cells than the contralateral testicle.

Similar work by Petrovitch et al. (1953) presented evidence of local effects of intratesticular pituitary grafts. Pituitaries were homografted in intact, immature guinea pigs. Interstitial cells hypertrophied for prolonged periods following transplantation. However, a lack of FSH secretion was indicated by retardation of spermatogenesis similar to that in the contralateral testes. Aron et al. (1956) divided the anterior pituitary tissue from guinea pigs into the acidophilic zone which was taken from the anterior border and the basophilic zone which was taken from the posterior border to the extreme anteroposterior median axis. Immature, nonhypophysectomized male guinea pigs received intratesticular homotransplants from these two zones. Most animals were autopsied 6 to 8 weeks later. Generally, the two types of grafts retained characteristics of their origin, but both types caused

local interstitial cell hypertrophy and stimulation of the seminal vesicles. The authors concluded that the staining characteristics of cells cannot be used to indicate the source of hormones.

Many other workers have presented evidence for gonadotropin secretion by ectopic pituitary grafts in male rats. A series of studies by Courrier and colleagues (Courrier 1956, 1957a, b; Courrier and Colonge, 1957 and Herlant et al., 1960) gave evidence of testicular maintenance in hypophysectomized rats bearing homografts of anterior pituitary tissue. The grafts were placed beneath either the kidney capsule or the membrane covering the isthmus between the lobes of the thyroid. Rats were autopsied 1 to 10 months after transplantation. The testicles were maintained in a functional state as indicated by the presence of spermatozoa in the seminiferous tubules. Testicular weight approached that of intact controls. Unilateral castration resulted in compensatory hypertrophy of the remaining testicle; a finding confirmed by Beddow and McCann in 1969. In the work of Courrier and Colonge (1957) and Herlant et al. (1960) the grafts contained poorly granulated gonadotropes and a massive hyperplasia of the cells of lactation. Herlant et al. (1960) reconciled the absence of typical gonadotropes by speculating that hypersecretion caused degranulation of these cells.

The kidney capsule was once again used by Ahrén et al. (1962) as a site for the autotransplantation of the pituitary gland of male rats. Fifteen to 16 months after surgery, three of the six experimental animals possessed testes and accessory sex organs similar to the two intact controls. The testes and accessory glands were atrophic in the remaining experimental animals. A more extensive study was reported by Smith and Davidson in 1967. One hundred eighty adult male rats, 60 to 90 days old had four pituitaries from 21 to 24 day-old donors grafted under the kidney capsule. The hosts were hypophysectomized 3 weeks after transplantation. Three weeks after hypophysectomy the grafted rats were unilaterally castrated. At this time only 8% of these animals had testicular weights equal to that of intact controls. These experimental animals had histologically normal testes, whereas the remaining 92% showed severe testicular atrophy. However, at 7 to 8 weeks after hypophysectomy the weight of the remaining testicle, seminal vesicles and ventral prostate was intermediate to those graft bearing animals with maintained testicular weight and hypophysectomized controls. In work concerning growth in weanling hypophysectomized rats bearing four pituitaries beneath the kidney capsule, it was noted by Hertz (1959) that the gonads were larger than the hypophysectomized controls. Varying degrees of sub-

optimal testicular function were observed in 10% of the animals bearing these grafts.

Pituitary tissue was transplanted in the anterior chamber of the eye of hypophysectomized male rats by Goldberg et al. (1955), Goldberg and Knobil (1957), and Martinovitch et al. (1963). Goldberg et al. (1955) and Goldberg and Knobil (1957) used fetal pituitaries, which produced proliferating, highly differentiated grafts. The anterior lobe was composed of mostly chromophobes, but there were also sparsely granulated acidophils, and well granulated basophils. Indications of FSH and LH secretion from about 40% of the grafts were spermatogenesis, the capacity to fertilize females, stimulation of testicular interstitial tissue, and subsequent stimulation of the accessory glands.

Multiple grafts of immature rat pituitaries were used by Martinovitch and co-workers (Martinovitch and Pavić, 1960 and Martinovitch et al. (1963). The hypophysectomized hosts received three or four whole anterior pituitaries at several intervals until a response was observed. In the earlier work testicular function was maintained in 12 of 38 grafted rats. Eleven of these males sired litters. Testicular maintenance was observed in 70% of the grafted rats in the later study.

Females

One of the first indications that pituitary transplants retain the capacity to secrete hormones in the absence of neurovascular connections to the hypothalamus was a report of short term studies by Emery et al. in 1931. This work was based on the hypothesis that unilateral castration caused hypersecretion of the anterior pituitary gland, which in turn caused compensatory hypertrophy of the remaining gonad. Pituitaries from adult, intact, and unilaterally castrated males and females were grafted in a muscle in the hind legs of 25 day-old, nonhypophysectomized female rats. The hosts were sacrificed 4 days later. There were no significant differences in ovarian or uterine weights in hosts which received grafts from either normal or unilaterally castrated donors. In all cases, however, these parameters increased greatly as compared with those of hypophysectomized control animals. No descriptions of the grafts were given. In 1936 Emery tested the potency of pituitary grafts after several days of transplantation. Most pituitaries were transplanted into the peritoneal cavity of immature female rats, but a few were grafted in the muscle of a hind leg. After several days in the first host grafts were removed and implanted into a second host. Twenty-one of 25 second recipients gave a positive vaginal response. With the exception of two animals all second

recipients having a positive vaginal response showed no increase in ovarian weight. When grafts remained in the first host for longer than 8 days a positive vaginal response was absent in the second recipient. However, histological examination of similar grafts indicated that this tissue was maintained for a much longer time. It should be considered that these observations may have resulted from a leaching of hormones rather than an active secretion from the graft.

Implants of anterior pituitary tissue were made subcutaneously in female mice by Hill (1935). Most implants were single pieces while others were composed of many fine pieces of tissue. No pituitary tissue was recovered from these later transplants. Examination of the ovaries at autopsy indicated a secretion of gonad-stimulating substance from the pituitary transplants. The work of Martins (1936), mentioned previously, also indicated gonadotropin secretion from pituitary homografts in female rats.

In 1937 May used only two hypophysectomized female rats to investigate the secretory capacity of intraocular pituitary transplants. Grafts were made 4 weeks after hypophysectomy. Within several days hair coat and body growth were restored and estrous cycles began 8 to 10 weeks later. One female was mated and gave birth to young. However, the completeness of hypophysectomy in one of these animals was

doubtful. Schweizer et al. (1937) placed intraocular pituitary homotransplants at the pupillary margin of the iris and in the lateral subconjunctival tissue in ten adult female hypophysectomized guinea pigs. These animals were studied for 3 to 13 weeks. In two animals follicular development increased for about 2 months and then constant estrus was observed. This follicular activity caused a constant estrous vaginal smear and a hypertrophy of the uterus with endometrial proliferation, indicative of abundant estrogen secretion. The mammary glands also were proliferated. The lack of fully mature follicles, ovulation, and subsequent corpus luteum development indicated that the grafts did not secrete luteinizing hormone. The cells of the pituitary grafts were predominantly basophilic.

Ovarian response to human chorionic gonadotropin (HCG) was used as a measure of gonadotropin secretion of pituitary grafts by Hertz (1960). Hypophysectomized female rats bearing four anterior pituitaries beneath the kidney capsule exhibited a significantly greater increase in ovarian weight in response to HCG than that of the hypophysectomized controls. Hertz believed the ectopic pituitary was capable of secreting enough gonadotropin to augment the effect of the HCG. Work by Baird et al. (1961) supports this observation. In immature female rats, hypophysectomized on the 6th day of pseudopregnancy, ovarian weight declined over a 5 week

period, but ovarian ascorbic acid levels were not depleted. After a week or more following hypophysectomy intravenous injection of LH produced no decrease in ovarian ascorbic acid. In another group of animals the pituitary was auto-transplanted to the kidney capsule on the 6th day of pseudo-pregnancy. Ovarian weight was maintained for 4 weeks; at which time there was a significant decrease in ovarian ascorbic acid. Intravenous injection of LH in these animals depleted ovarian ascorbic acid to lower levels than that of intact pseudopregnant rats. The previously discussed work of Martinovitch et al. (1966) is additional evidence for the ability of ectopic pituitary tissue to secrete gonado-tropins after prolonged periods of cultivation in vitro.

Gittes and Kastin (1966) have demonstrated that the amount of tropic hormones produced by ectopically placed pituitary tissue must be at a fixed level. Hypophysectomized weanling female rats received intramuscular homotransplants of 1, 3, 10, or 30 pituitaries. The growth of these grafted animals was proportional to the logarithm of the number of grafted glands. Ovarian and uterine weights also increased with increasing numbers of pituitary grafts. In the rats grafted with 30 glands the ovaries contained mature follicles and numerous corpora lutea. From the estimated activity of a single graft it was extrapolated that many hundreds of grafts would be required to support normal growth in these

hypophysectomized rats, but 10 or 30 of these grafted pituitaries produced highly significant gonadotropic activity.

Evidence Against Gonadotropin Secretion by Ectopic Adenohypophysial Transplants

Males

In a study of adrenocorticotropin secretion from anterior pituitary grafts, Cheng et al. (1949) noted extreme atrophy of the testes of hypophysectomized rats bearing an intraocular or intraspleenic adenohypophysial graft. Martini et al. (1959) homografted pituitaries into the anterior chamber of the eye of adult male rats. The rats were hypophysectomized 203 days after surgery. The testes of the grafted rats atrophied to the same degree as hypophysectomized, nongrafted animals.

Females

Absence of gonadotropin secretion by anterior pituitary grafts was demonstrated by Richter and Eckert in 1937. The grafts were pituitaries from immature rats placed into the anterior chamber of the eye 10 to 20 days after hypophysectomizing the female hosts. There was no ovarian stimulation by 8 to 18 days later. The anterior chamber of the eye was used by Westman and Jacobsohn (1940) for homotransplantation of pituitaries from mostly young female rats to 28 adult or immature, hypophysectomized rats. There were no

vaginal cycles observed for 5 months and ovaries were completely atrophied. Further evidence of a failure of pituitary grafted tissue to secrete gonadotropins was noted by Greer et al. (1953) in a study concerning thyrotropin secretion of intraocular grafts of mouse pituitary tissue. Ovarian and uterine weights were not significantly different from the hypophysectomized controls. A later review by Greer (1957) also pointed out the failure of anterior pituitary grafts to secrete gonadotropin in mice.

Everett (1954 and 1956a), Sanders and Rennels (1957 and 1959) and Rothchild (1960a) investigated the activity of anterior pituitary autografts in adult and prepubertal rats. The tissue was grafted beneath the kidney capsule. After 4 months the condition of ovarian follicles and interstitial tissue indicated an absence of FSH and LH production by the pituitary grafts. Similar observations in rats bearing adenohypophysial grafts beneath the kidney capsule and temporal lobe of the brain were reported by Nikitovitch-Winer and Everett (1957a, b) and Stolzenberg (1969).

In 1957 Ifft grafted pituitary tissue from newborn hamsters into the cheek pouch of adult females. The host animals were either completely hypophysectomized or possessed small fragments of pituitary tissue left in the sella turcica. There were no estrous cycles observed in the completely hypophysectomized hosts. However, a slight indica-

tion of gonadotropin production of the pituitary graft was noted due to an alteration of the vaginal smear cycles of the incompletely hypophysectomized hosts after removal of the grafts.

Van Rees and Wolthius (1962) reported that anterior pituitaries homografted beneath the kidney capsule of non-hypophysectomized rats rapidly lose their FSH content. Administration of testosterone inhibited this decrease in FSH content in the graft and in some cases even increased it. Progesterone had no effect, but estradiol was antagonistic to the effect of testosterone on the FSH content of the pituitary grafts.

The literature discussed here has presented evidence for all degrees of gonadotropin secretion by anterior pituitary tissue grafted to sites remote from the central nervous system. In most cases this secretion appeared to be below normal, suggesting that normal gonadotropic stimulation requires a close relationship of the adenohypophysis with the central nervous system.

Luteotropin or Prolactin Secretion by Ectopic Adenohypophysial Transplants

Maintenance of the corpus luteum

According to Greenwald and Rothchild (1968), Desclin (1950) was the first to demonstrate the capacity of the transplanted pituitary to secrete prolactin. A discussion

of this work by Everett (1950) and Greenwald and Rothchild (1968) revealed that Desclin treated his experimental animals bearing a pituitary autotransplant with stilbesterol. Controls, which were not treated with the estrogen, were not included. Desclin concluded that the presence of the estrogen was necessary for prolactin secretion by the ectopic pituitary. Earlier studies have demonstrated that estrogens do influence the morphology and secretion of grafted anterior pituitary tissue. Martins (1936b) found that both the *in situ* pituitary and ectopic pituitary placed in the anterior chamber of the eye of rats possess decreased numbers of basophils and increased numbers of chromophobes after administration of estrone. At the same time Desclin and Grégoire (1936) demonstrated that exogenous estrogen in rats bearing homotransplanted pituitary tissue caused the appearance of numerous small basophils and a significant increase in the number and size of acidophils. Since these grafts previously contained less than normal numbers of acidophils, the authors concluded that sex hormones act directly on the pituitary gland. In 1939 Phelps *et al.* found that estrogen treatment of female rats bearing pituitary grafts in the thigh muscle influenced the survival and structure of the grafts. The anterior lobe degenerated after transplantation but then underwent regeneration. The survival rate of the grafts and the degree of regeneration were increased by the estro-

gen treatment. The grafts of uninjected hosts contained small numbers of chromophils; whereas, those of injected hosts possessed an increase in the number of chromophils. Another investigation by Desclin and Koulischer in 1960 demonstrated an estrogenic influence on grafted pituitary tissue. The prolactin content and cellular morphology of pituitary tissue grafted to the kidney capsule of female rats were studied. Estrogen treatment increased the prolactin content of the grafted tissue. A later study by Wolthius and deJongh (1963) has further demonstrated stimulation of pituitary grafted tissue by estrogen. Prolactin secretion of pituitary grafts beneath the kidney capsule of hypophysectomized females was compared with that of in situ pituitaries in intact females. Counts of luteal cell nuclei were used as the end point of measurement. Small amounts of estrogen in the peripheral circulation increased the prolactin effect of both the hypophysial grafts and the in situ pituitaries. Another indication of these effects was observed when the prolactin content of serum and pituitary grafts was assayed in pituitary grafted animals which were either ovariectomized or intact. The prolactin content of the grafts was lower and the prolactin levels of the serum were higher in the ovariectomized animals as compared with the intact animals.

A series of studies by Everett (1954, 1956a, b) and

Nikitovitch-Winer and Everett (1958a) demonstrated that estrogen was not essential for the secretion of high levels of prolactin or luteotropin by ectopic pituitary tissue. The pituitary gland was autotransplanted beneath the kidney capsule on the day after ovulation. The end point of measurement for corpus luteum function was formation of deciduomata by the traumatized uterus. The uteri were traumatized 4 days after transplantation and the animals were autopsied on the 8th day. The control rats in which hypophysectomy had either been complete or nearly complete failed to form deciduomata. Deciduomata had formed in grafted animals in which hypophysectomy had either been complete or nearly complete. Some nongrafted rats which retained large fragments of pituitary tissue in the sella turcica gave a significant decidual reaction. In a later investigation Everett (1956a, b) used vaginal mucification following the administration of an excess of estrogen as a test for function of the corpus luteum. The estrogen was given the last week of the experimental period, which ranged from 23 to 120 days. Corpora lutea were maintained at a diameter of 1.5 mm and the vaginal epithelium was atrophied in grafted rats killed at 26 to 90 days. These animals did not receive an injection of estrogen. In other grafted rats given estrogen, the corpora lutea were enlarged to an average of 2.0 mm and the vaginal epithelium

was mucified at autopsy 23 to 104 days after transplantation. Removal of the graft, in a group of rats which responded to the estrogen treatment, resulted in a degeneration of corpora lutea to a diameter of less than 1.2 mm within 8 days. Ovarian follicles and interstitial tissue atrophied in these animals. Luteal function was tested by Everett (1956b) in pregnant rats bearing pituitary autotransplants. After a single injection of 0.1 µg of estradiol benzoate as early as 4 days or as late as 12 days after ovulation, implantation sites were present within 4 to 5 days. Four of 13 rats maintained pregnancy to term. The others resorbed their embryos either before or after the placentae were well established.

Recent work by Carpent and Desclin (1967) on maintenance of pregnancy in rats bearing pituitary autografts beneath the kidney capsule demonstrated that estrogens can stimulate implantation. Contrary to the observations of Everett (1956b), further maintenance of pregnancy was rare, even with continued injections of estrogen.

The decidual reaction of the uterus was used as an indication of secretion of luteotropin by autotransplants of the hypophysis by Nikitovitch-Winer and Everett in 1958a. The tissue was autotransplanted beneath the kidney capsule or into the anterior chamber of the eye. No estrogen secretion was evident in the pituitary grafted rats, but the

uteri reacted to traumatization for as long as 42 days. Neither the stage of the cycle at which transplantation occurred nor the site of the transplant influenced luteotropic activity of the graft. Sanders and Rennels (1957 and 1959) also observed maintenance of the corpus luteum by luteotropin secreted by pituitary autografts placed beneath the kidney capsule. The grafts contained chromophobes and acidophils, which stain selectively with orange G. The authors suggested that the acidophils were the source of luteotropin in the rat.

Pituitary transplantation was used to provide constant secretory levels of luteotropin by Rothchild (1960a and 1965) and Rothchild and Schwartz (1965) to study mechanisms of luteal maintenance in rats. Neither unilateral castration nor administration of progesterone affected the size of corpora lutea in pseudopregnant or pituitary autografted animals (Rothchild, 1960b). It was suggested that the progesterone-luteotropin relationship was not a negative feedback mechanism. Rothchild (1965) demonstrated a luteolytic effect of LH in adult rats bearing hypophysial autotransplants. Beginning 2 weeks after transplantation, LH was given for 10 or 24 days. Ten days of LH treatment caused a decrease in the size of the corpora lutea and the degree of progesterone secretion, which were directly related to the dose of LH. Progesterone secretory activity

was determined by the mucification of the vagina and proliferation of the uterus after 3 daily injections of estradiol benzoate. In pituitary autotransplanted controls and similarly prepared rats given LH, the corpora lutea were larger after injections of estrogen than found in uninjected animals. Rothchild and Schwartz (1965) then attempted to determine if the presence of LH or the absence of luteotropic hormone (LtH) was the cause for luteal regression, and, if the presence of LtH or the absence of LH caused luteal maintenance. The corpora lutea of pseudopregnant control rats were in advanced stages of regression by 28 days after ovulation. When pseudopregnant rats were treated with progesterone or a combination of progesterone plus a pituitary homotransplant, luteal regression occurred at the same time. The presence of follicular growth, vaginal mucification, and pituitary LH content equivalent to cyclic rats at proestrus indicated maintenance of basal levels of LH secretion. As observed in previous work, progesterone failed to induce luteal regression in rats bearing an autotransplant of the pituitary gland. Increasing the dosages of estradiol benzoate in pseudopregnant rats for a period of 28 days resulted in luteal maintenance in 89% of the animals. The secretion of LH was markedly reduced with even the lowest dose of estrogen (5 μg/day). Unlike progesterone treatment, exogenous estrogen in pseudopregnant rats bearing one or

two pituitary homotransplants caused maintenance of the corpus luteum. A combination of estradiol benzoate and progesterone for 28 days maintained luteal growth in almost all rats. Secretion of endogenous LH in rats given estrogen and progesterone declined as much as those given only estrogen. Exogenous LH induced luteal regression in rats injected with estrogen plus progesterone. Exogenous estrogen and progesterone failed to maintain corpora lutea in hypophysectomized rats. These results indicate that for luteal maintenance it is essential to have the presence of LtH and an absence of LH. Zeilmaker (1964) studied luteal function in androgen sterilized female rats. The morphology of the vaginal epithelium and the distribution of sudanophilic material in the luteal cells were used to characterize possible function of induced corpora lutea. Pseudopregnancy could not be induced by daily cervical stimulation, by 3 daily injections of reserpine, or by the presence of a pituitary homotransplant beneath the kidney capsule during the first 4 days after induction of corpora lutea. It was concluded that a "tonic LH secretion" by the in situ pituitary produced luteal regression. However, luteal function could be maintained for at least 45 days by the continual presence of a pituitary graft.

Choudary and Greenwald (1967) used pituitary homotransplants beneath the kidney capsule in hypophysectomized and

intact hamsters to study the luteotropic complex of FSH and LtH. In previous work by Turnbull and Kent (1966) and Greenwald (1967) these gonadotropins were injected into hypophysectomized hamsters for maintenance of the corpus luteum. Luteal maintenance was evaluated by ovarian histology and decidual reaction (Choudary and Greenwald, 1967). Homografts of single anterior pituitaries beneath the kidney capsule in cycling, intact hosts induced repeated pseudopregnancy-like cycles of 8 to 10 days. Pseudopregnancy could not be stimulated by a pituitary homograft in day 3 or 4 recipients, but subsequent cycles were of the pseudopregnancy type in this group. In pregnant animals which were hypophysectomized and given replacement therapy, only administration of both FSH and LtH maintained pregnancy. In pregnant and pseudopregnant, hypophysectomized hamsters bearing a pituitary homotransplant, only exogenous FSH was needed to maintain pregnancy or a decidual response. A similar, but limited, study was reported by McDaniel et al. (1967). One, four or six anterior pituitaries were homotransplanted to the cheek pouch of nonhypophysectomized female hamsters which were either intact or hysterectomized several weeks before pituitary transplantation. Six pituitary grafts were necessary to consistently increase the duration of diestrus of either intact or hysterectomized hamsters to an average of 8.7 and 17.7 days, respectively.

The first prolonged diestrous interval occurred on the average of 15 days after transplantation. When the transplants were removed from host animals estrus occurred 1 to 3 days later, indicating that continuous secretion of luteotropin by the pituitary homografts was essential for prolonged luteal maintenance. It was concluded by both groups of workers that the pituitary transplants in intact cycling hamsters induced pseudopregnancy cycles by producing the LtH component of the luteotropic complex.

Many workers have investigated the influence of pituitary homografts on the luteal function of pituitary-intact hosts. In 1958, Alloiteau autotransplanted one half of the pituitary gland of cycling female rats to the kidney capsule. The other half remained in situ. Regular estrous cycles were replaced with repeated pseudopregnant cycles. Similar results were obtained by Quilligan and Rothchild (1960) and Silbiger and Rothchild (1963). Quilligan and Rothchild (1960) studied the effect of a pituitary homotransplanted beneath the kidney capsule on the estrous cycle of intact rats. Control rats were homotransplanted with cerebellar tissue. Most of the pituitary grafted animals showed a pseudopregnancy-like prolongation of diestrus which was in progress at the time of the homotransplantation. Subsequent cycles tended to be prolonged. As in the hamster (Choudary and Greenwald 1967), those rats grafted in late diestrus

(day 2) failed to show a prolonged cycle, but succeeding cycles were prolonged. There was a high incidence of incomplete estrous smears, characterized by only a single day of nucleated or cornified epithelial cells, which were preceded and followed by smears of predominantly leucocytes. The control animals maintained normal estrous cycles.

In an investigation on the influence of the uterus on the corpus luteum-pituitary relationship in rats, Silbiger and Rothchild (1963) observed that hysterectomy of pseudopregnant rats caused a prolonged diestrous interval. If hysterectomy were performed before the late afternoon of the 9th day of pseudopregnancy 78% of the animals showed prolonged pseudopregnancy, but hysterectomies from day 9 to day 12 resulted in prolonged pseudopregnancy in less than 40% of the animals. Normal estrous cycles returned after the pseudopregnant cycle. The placement of a pituitary homotransplant beneath the kidney capsule of pseudopregnant rats at the time of hysterectomy did not increase the percentage of prolonged pseudopregnancies of the first post-operative cycle, but did increase slightly the diestrous interval. There was a 46% increase in the incidence of all pseudopregnancy cycles compared with intact pseudopregnant rats bearing a pituitary homotransplant. Ten percent of the pseudopregnancy cycles were prolonged in the intact animals and 44% were prolonged in the hysterectomized animals.

The responses of ovarian homografts to exogenous gonadotropins and to pituitary homografts were studied in pituitary-intact, cycling female rats by Browning and Guzman in 1967. Ovarian and pituitary homotransplants were placed in the anterior chamber of the eye. Ovarian grafts in gonad-intact females developed only primordial follicles, which did not respond to exogenous FSH. Ovarian grafts in ovariectomized animals developed vesicular follicles and corpora lutea which in turn stimulated normal vaginal smear cycles. New corpora lutea were hyperemic for approximately 2 days. Exogenous LH did not affect luteal hyperemia or vaginal cycles. Administration of prolactin to ovariectomized, ovarian-grafted rats for 20 to 28 days maintained corpora lutea during the treatment period, as well as 2 to 17 days beyond the last injection of prolactin. The pituitary graft in ovariectomized, ovarian-grafted rats caused continual development of hyperemic corpora lutea, which were maintained more than 3 months. A diestrous vaginal smear was prolonged and a decidual response could be elicited by the uteri. In work concerning mammotropic effects of pituitary grafts, Dao and Gawlak (1963) noted that corpora lutea of ovarian transplants in hypophysectomized, ovariectomized, pituitary-homotransplanted rats were maintained for prolonged periods.

An extensive series of studies on the luteotropic

influence of intraocular or subcutaneous pituitary homografts in nonhypophysectomized mice has been reported by Browning and White (1960, 1962, 1963, 1965a, b) and Browning (1964). Ovariectomized mice bearing bilateral homografts of ovarian tissue in the anterior chamber of the eye were used in three of these studies (Browning and White, 1960, 1962, 1963). These ovarian grafts developed mature follicles and pale corpora lutea in a cyclic manner. As in similarly prepared rats (Browning and Guzman, 1967) luteal function lasted approximately 2 days. When a pituitary graft was placed within the anterior chamber of one eye already containing an ovarian homotransplant, pseudopregnancy-like vaginal cycles occurred with the development of hyperemic corpora lutea. Unlike similarly prepared rats (Browning and Guzman, 1967), in which new corpora lutea were maintained for months, luteal hyperemia lasted for approximately one-half the length of pseudopregnancy. The diestrous interval increased with increasing numbers of pituitary grafts (Browning and White, 1963). In rats possessing 16 pituitary grafts, vaginal cycle length was approximately 22 days and luteal hyperemia lasted about 16 days. When intact male rats received intraocular ovarian and pituitary homografts (Browning, 1964 and Browning and White, 1965b), corpus luteum formation occurred sporadically. Luteal hyperemia lasted 2 or 3 days. Exogenous LH stimulated the formation of

hyperemic corpora lutea in 70 to 90% of the ovarian grafts. This luteal hyperemia lasted from 7 to 12 days, depending on the number of injections of LH and the number of pituitary grafts.

Boot et al. (1959) transplanted pituitaries beneath the kidney capsule, in the spleen, subcutaneously, intraperitoneally and within the ovarian bursa in intact, female mice. Most animals were grafted at 8 weeks of age. Pseudopregnancy-like estrous cycles were observed. Transplantation of a large number of pituitaries caused an overlapping of the progestational phases of successive psuedopregnancies until a continuous diestrous vaginal smear was observed. Similar effects of pituitary homografts on the estrous cycle of intact mice were observed by Dominic (1966) and Hagen and Rawlinson (1963).

An indirect method for demonstrating constant secretion of prolactin (luteotropin) by ectopic pituitary tissue is the maintenance of delayed implantation. Autotransplantation of the pituitary glands of female rats several days after mating resulted in delayed implantation of embryos for as long as 18 days (Meyer et al., 1958; Meunier and Mayer, 1961 and Cochrane et al., 1962). In 1960 Prasad et al. found that only progesterone was essential for implantation in the hamster. Implantation did not occur in hamsters bearing pituitary autotransplants. This was probably caused

by absence of FSH; a gonadotropin necessary for the luteotropic complex (Choudary and Greenwald, 1967).

The work discussed here demonstrates the inherent capacity of the anterior pituitary gland of laboratory animals to secrete luteotropin (prolactin) when removed from the inhibitory influence of the central nervous system. In many instances there is a hypersecretion of the tropic hormone. Exogenous prolactin under certain conditions provides a luteolytic influence (Malven, 1965; Malven and Sawyer, 1966 and MacDonald and Greep, 1969). Luteal regression was markedly accelerated and ovarian weight was significantly decreased in adult rats given prolactin beginning several weeks after hypophysectomy. Malven and Sawyer (1966) postulated that within a few days after hypophysectomy the corpora lutea lose the capacity to be maintained by prolactin and once this occurs, exogenous prolactin induces luteolysis. In 1969, Sloan and Malven homografted pituitaries from rats at various stages of the estrous cycle into female rats 12 days after hypophysectomy. The host animals were examined 5 or 8 days later. The presence of the pituitary graft caused decreased ovarian weight and structural lysis of the corpora lutea. This luteolysis was equivalent to that produced by exogenous prolactin in non-grafted, hypophysectomized females. This work further demonstrates the capacity of ectopic pituitary tissue to

maintain high levels of prolactin secretion. Stolzenberg et al. (1969) autotransplanted the pituitary gland beneath the kidney capsule of 11 to 12 week-old female rats during metestrus, whereas control animals were hypophysectomized. Exogenous estrogen at various dosages and times following surgery produced an immediate increase in ovarian weight, but long term treatments caused a decline in ovarian weight due to regression of the corpora lutea. Estrogen produced no effect on ovarian weight in hypophysectomized, non-grafted rats, indicating the necessity of the pituitary tissue for this response. Stolzenberg et al. (1969) proposed that the estrogen could have stimulated an increased secretion of prolactin, a temporary surge in LH secretion from the pituitary graft, or a direct action on the ovary. Vasopressin and oxytocin were used by Stolzenberg et al. (1968) to modify the secretion of pituitary autografts placed beneath the kidney capsule in mature rats the day after estrus. Ovarian weight decreased after administration of vasopression for 45 or 70 days and after administration of oxytocin for 70 days. Similar treatment of hypophysectomized, nongrafted rats failed to produce this effect.

Development of mammary glands

Because it is widely accepted that prolactin and luteotropin are the same hormone in most laboratory animals, development and function of mammary glands are used to assess

secretion of prolactin by ectopic pituitary tissue. The early work by Loeb and Kirtz (1939) dealt with the function of ectopic pituitary tissue. The experimental animals were intact strains of mice with high and low incidences of spontaneous mammary gland carcinoma. They received three or four anterior pituitary glands subcutaneously. After 8 to 10 months the ectopic pituitaries stimulated mammary gland growth and secretory activity in both strains of mice. There was also an increase in the development of mammary gland carcinoma in the strain with the high incidence of spontaneous mammary cancer. However, mammary gland cancer was not stimulated by the transplanted pituitary tissue in the strain with the low incidence of spontaneous mammary cancer. These effects were absent in ovariectomized mice. The authors proposed that active corpora lutea were necessary to promote the influence of the pituitary graft, since large active corpora lutea were present in mice bearing pituitary homografts. Later work by Loeb et al. (1944), and Silberberg and Silberberg (1949 and 1950) demonstrated that mammary gland carcinoma was induced in castrated male mice by subcutaneous transplants of ovarian and pituitary tissue. Mammary gland development was stimulated by pituitary grafts alone. Castration was essential for these responses to occur. The experimental animals were from a strain which had a low incidence of spontaneous mammary gland cancer.

In 1958 Liebelt reported that intraocular pituitary grafts in intact virgin female mice, possessing the mammary tumor agent, inhibited the growth of spontaneous or transplanted mammary cancers. Similar results were obtained in intact, lactating mice which had no pituitary transplants. Mammary gland development was observed in the virgin mice in 50 to 60 days after hypophysial transplantation. Liebelt and Liebelt (1961) later found mammary gland stimulation with milk secretion and formation of functional corpora lutea in intact female mice which received either a subcutaneous or intraocular pituitary homotransplant. The mice used were strains which either possessed or lacked the mammary tumor agent. Contrary to the results of Liebelt (1958) mammary cancer was induced in both mice with or without the mammary tumor agent. Many other workers have reported the induction of mammary gland carcinoma by single or multiple pituitary homografts in mice (Boot et al., 1959, 1960, 1962; Halberg et al., 1959; Mühlbock and Boot, 1959; Dux and Mühlbock, 1969). Boot et al. (1959 and 1960) found that pituitary grafts from male donors stimulated a greater percentage of mammary tumors than those from female donors.

In 1957 Desclin found that reserpine stimulated lactation in intact virgin female rats. Reserpine also stimulated lactation in hypophysectomized virgin females bearing a pituitary transplant beneath the kidney capsule, but failed to

stimulate lactation in nongrafted, hypophysectomized females. Desclin (1957) concluded that reserpine either synergized with or stimulated the secretion of prolactin by the grafts.

Several workers have used female rats, which already had mammary glands developed to the secretory stage, to study prolactin secretion of pituitary grafts. Meites and Hopkins (1960) used 10 μg of estradiol benzoate daily for 10 days to develop the mammary glands of mature female rats. Homografts of pituitary tissue in the renal capsule of intact or hypophysectomized rats induced lobular-alveolar growth, and in some animals, milk secretion. The mammary glands of control rats regressed to a bare duct system and produced no secretion. Cowie et al. (1960) used pregnancy to develop mammary glands in their experimental animals. Female rats, which had received pituitary homotransplants from their 7 or 8 day-old pups of the previous gestation, were hypophysectomized on day 4 of lactation. The grafts were placed beneath the kidney capsule or within the anterior chamber of the eye. Only slight and temporary milk secretion was maintained in the grafted rats. Daily injections of adrenocorticotropic hormone (ACTH) increased the amount and duration of milk secretion in the hypophysectomized graft bearing rats. Similar results were obtained by Ahmad and Lyons (1966), except they used prednisolone acetate to supply glucocorticoid rather than

ACTH. If the pituitaries were autotransplanted to the kidney capsule during lactation and 0.5 units of oxytocin were injected every 4 hours without the exogenous corticoid, lactation was maintained at about 50% that of the control animals. Rothchild (1960a) reported the maintenance of lactation in rats which had their pituitaries autotransplanted beneath the kidney capsule 4 to 6 days postpartum. The survival and weight gain of the pups increased with increased frequency of oxytocin treatment of the mothers. All litters in which the mother received no oxytocin died within 6 days. Histological sections of mammary tissue indicated that 50 to 80% of each gland showed active secretion.

The secretory capacity of transplanted pituitaries has been studied by Ahrén (1961) using gonadal steroids to support mammary gland development in castrated male and female rats bearing a pituitary autotransplant under the kidney capsule. Pituitaries were autotransplanted following castration and later exogenous hormones were given. Daily administration of 10 µg of estrone alone or in combination with 4 mg of progesterone stimulated slight duct growth and lobular-alveolar development in rats bearing pituitary autotransplants. Such treatment in rats having their pituitary gland in situ stimulated extensive mammary gland development, but failed to stimulate such development in hypophysectomized, nongrafted rats. However, if prolactin were

combined with the estrone and progesterone treatment in hypophysectomized, nongrafted rats, mammary gland development was stimulated to the same degree as in rats bearing a pituitary autotransplant.

The local effects of subcutaneous adenohypophysial homografts on mammary gland development have been studied by Dao and Gawlak (1963) and Meites and Kragt (1964). A single anterior pituitary was implanted near a mammary gland in hypophysectomized female rats which were either ovariectomized, ovariectomized and grafted with ovarian tissue, or intact. Growth of ducts and alveoli occurred near the pituitary transplant, whereas the glands opposite the transplant remained atrophied. These workers concluded that the ectopic pituitary tissue synthesized and released significant quantities of prolactin and that the observed mammary gland development did not require the presence ovarian or adrenal cortical hormones.

Many workers have used the pituitary intact mouse as an experimental animal to study the mammotropic influence of ectopic pituitary tissue. Bardin et al. (1964) homotransplanted a single pituitary gland to the anterior chamber of the eye of intact virgin mice. These grafts stimulated lobular-alveolar growth in intact mice, but not in hypophysectomized mice. The pituitary grafts maintained only partial lobular-alveolar development if the intact hosts

were later hypophysectomized. They concluded that there was an interdependence between the pituitary graft, the pituitary gland *in situ*, and the ovaries. The local effect of pituitary grafts on mammary gland development in pituitary intact mice was studied by Bardin *et al.* (1962) and Bardin and Liebelt (1961). Bardin and Liebelt (1961) again found that intraocular pituitary homografts in females and males stimulated growth of mammary ducts and alveoli. These workers also homografted pituitary tissue to a site near the mammary gland in gonad-intact males and females, and in ovariectomized females. In the gonad-intact mice, lobular-alveolar and ductal development was greater near the pituitary transplant than that found on the opposite side. The same results were obtained by Montemurro and Gardner (1963) using subcutaneous homografts of two pituitary glands placed between the third and fourth mammary glands on one side. Ovariectomy reduced this development (Bardin and Liebelt, 1961). Contrary to similar work with rats (Dao and Gawlak, 1963 and Meites and Kragt, 1964), these results indicate that the ovary was necessary for maximal response of mammary tissue to the local effect of a pituitary graft.

In a study by Browning and White (1965a) mice were ovariectomized and had ovarian homografts placed subcutaneously at the tip of the fourth mammary gland on one side. A group of these mice also received a pituitary homotrans-

plant either adjacent to the same gland or on the opposite side. After 3 months there was alveolar development near the ovarian tissue and similar development plus secretion around the pituitary grafts. This response was enhanced when the ovarian and pituitary grafts were together. Intact mice without ovarian grafts or pituitary grafts possessed no alveoli in their glands; whereas, those with only a pituitary graft showed alveolar proliferation and secretion in the gland containing the pituitary transplant. Browning et al. (1963) prepared ovariectomized mice with intraocular ovarian homografts. Groups of these ovarian grafted mice received, 0, 1/32, 1/8 or 1/2 of an anterior pituitary in the anterior chamber of the eye. Other groups received 1/2, 2, 8 or 16 anterior pituitaries subcutaneously. After 8 months all mice with pituitary transplants except a few with 1/32 showed alveolar development and secretion with extremely dilated ducts. Mammary gland development in intact controls remained unchanged, but that of the ovariectomized, ovarian grafted mice without transplanted pituitary tissue was similar to those animals having pituitary transplants. Hyperplastic nodules were observed in increasing numbers as the amount of graft increased from 1/8 of an anterior lobe to 16 lobes.

Anterior pituitary transplantation has been used to study the control of prolactin by the in situ pituitary in

rats. Mena *et al.* (1968) homografted one, four or eight adult rat anterior pituitaries or ten anterior pituitaries from immature rats to the kidney capsule on days 12 through 16 of pregnancy and measured the prolactin content of the *in situ* anterior pituitary on days 7, 14 or 21 of lactation. In a similar study Sinha and Tucker (1968) homografted two, five or ten anterior pituitaries from adult rats to the kidney capsule of mature virgin rats. One group which received ten anterior pituitaries was ovariectomized. The prolactin content of the *in situ* anterior pituitaries was measured after 14 days. Both groups of workers found that the transplanted pituitaries caused decreases in prolactin content of the *in situ* pituitaries ranging from 67 to 91% that of the control levels. Sinha and Tucker (1968) reported that anterior pituitary transplants caused an increase in total mammary DNA and RNA, but this tended to be reduced by ovariectomy. Ovariectomy appeared to cause a further decline in prolactin content of the *in situ* pituitary gland. Both groups of workers concluded that sufficiently high levels of circulating prolactin inhibited further secretion of prolactin by the anterior pituitary and that the presence of the ovary was not essential for this response.

Hypopthalamic Control of the Adenohypophysis

Early evidence that the adenohypophysis must be in close relationship to the central nervous system for normal activity was presented by Greep in 1936. Pituitary auto- and homo-transplants were made into the empty sella turcicas of 28 day-old male and female rats. Grafts were successful in 73% of the 37 pituitary grafted rats. Body growth was stimulated from 1/2 to 2/3 that of intact controls and sexual activity resumed in both males and females. Estrous cycle length was 4 to 14 days. Thirteen pregnancies were observed with delivery of normal young. Testicular function was returned to pituitary grafted males. The sex and age of the donor had no effect on observed results. Similar results were obtained in later work by Harris and Jacobsohn (1951 and 1952a, b). Pituitary homografts from immature, adult male and female rats were implanted under the median eminence, under the temporal lobe of the brain, or into the empty sella turcica of hypophysectomized hosts. Grafts became well vascularized in all three sites. Those grafts under the median eminence were vascularized by the primary plexus of the hypophysial portal vessels; whereas those under the temporal lobe and within the sella turcica acquired vascular connections with other vessels. Only grafts under the median eminence showed normal activity as indicated by the occurrence of estrous cycles, maintenance of pregnancy,

parturition and maintenance of the adrenal glands. Grafts in the remaining sites showed little or no activity. These workers concluded that anterior pituitary secretion was under hypothalmic control, mediated by the hypophysial portal vessels. Another series of similar work has been reported by Smith (1959, 1961, 1963). Pituitary homotransplants from adult male and female donors were inserted in the sella turcica of adult male and female rats which had been hypophysectomized 21 to 375 days previously. Animals were killed 75 or 125 days after pituitary transplantation. With the exception of a few animals, growth, thyroid, adrenal, and sexual activity resumed. Regular estrous cycles of 4 to 6 days were observed. Both male and female hosts became fertile. Most females which were mated gave birth to young, but lactation was deficient or absent. Cutuly (1941) hypophysectomized male rats and autotransplanted pituitary tissue in the anterior chamber of the eye or within the empty sella turcica. Varying degrees of stimulation of both the seminiferous tubules and interstitial tissue of the testes and subsequent stimulation of the seminal vesicles were observed in all animals. Two fertile matings from a rat with an ocular transplant and one fertile mating from a rat with a sella turcica graft were observed about 200 days after transplantation.

Nikitovitch-Winer and Everett (1957a, b; 1958b and 1959)

sought to answer the question of whether the cytological and functional changes of transplanted adenohypophysial tissue were due to operative trauma and ischemia, or the loss of the normal vascular connections with the hypothalamus. The anterior pituitary was autotransplanted beneath the kidney capsule in adult female rats. Two to 4 weeks later these grafts were removed from the kidney capsule and placed either beneath the median eminence or the temporal lobe of the brain. When the retransplanted tissue was in close contact with the median eminence, estrous cycles were observed in a large proportion of the animals 8 to 75 days later. Small daily doses of estrogen stimulated the return of regular estrous cycles in a group which remained anestrus after retransplantation of the anterior pituitary gland. Pregnancy could be maintained and the atrophied adrenals and thyroids were restored in these cyclic animals. In those animals in which the tissue was retransplanted beneath the temporal lobe, the reproductive tract, adrenals, and thyroids remained atrophied. These animals continued to be anestrus. There was considerable necrosis of the adenohypophysial autografts in the kidney capsule as indicated by a thin shell of functional tissue surrounding a massive infarct. Small basophils persisted for several weeks but thereafter they were rarely observed. Small chromophobes were the predominant cell type in these auto-

grafts. Further necrosis of the autograft was evident soon after the second transplantation. Those grafts placed beneath the temporal lobe were similar to those beneath the kidney capsule, however, grafts placed beneath the median eminence recovered normal cytological characteristics. There was a return of large gonadotropes with castration cells predominant in those animals which remained anestrus. Large thyrotropes were also abundant.

Further evidence that the secretory activity of the anterior pituitary is controlled by the central nervous system and not within the pituitary gland itself was presented by Martinez and Bittner in 1956. Hypophysectomized female and male mice received three male or female mouse pituitaries within the empty sella turcica. The female hosts showed normal estrous cycles whether the donor pituitaries were from males or females. The male hosts had been castrated and possessed grafts of ovaries and a ring of vaginal tissue. These grafted ovaries and vaginal tissue showed a continuous noncyclic type vaginal stimulation whether the donor pituitaries were from male or females. The authors concluded that sex differences in gonadotropin secretion did not depend on a difference in the pituitaries, but in some other controlling structure.

The specific area of the central nervous system which controls pituitary function has been more clearly defined

by transplanting pituitary tissue directly into various sites of the brain of the rat. In 1962, Knigge stereotaxically implanted pituitary glands of neonatal rats into various regions of the hypothalamic area of adult, hypophysectomized rats. Forty-four of 58 grafts were viable 3 months later. Thyrotropic and adrenocorticotropic activity were absent in all grafted animals. When the pituitary grafts were located in the floor of the hypothalamus, adjacent to or interrupting the fibers of the supraoptico-hypophysial tract testicular weight and function were maintained. These grafts possessed large vacuolated basophils with an absence of acidophils. When the pituitary grafts were located in the preoptic area, the septal area, the anterior hypothalamus, or the mammillary body the testes atrophied. These grafts possessed small, uniform chromophobic cells. Halász et al. (1962) found similar results and designated the graft-stimulating region of the hypothalamus as the "hypophysiotrophic area". Further studies on pituitary grafts placed in the hypophysiotrophic area were reported in 1965 by Halász et al. and Falment-Durand. Pituitaries were homotransplanted into various parts of the brain in addition to the medial basal hypothalamus or hypophysiotrophic area in hypophysectomized adult male and female rats. In animals which had pituitary tissue within the hypophysiotrophic area, gonadotropic hormone secretion

was normal as evidenced by normal vaginal smears, mature follicles, fresh corpora lutea, and normal ovarian weight. Ovarian compensatory hypertrophy was observed upon unilateral ovariectomy. Grafts contained both acidophils and basophils. In animals which had pituitary tissue outside the hypophysiotrophic area the gonads, adrenals, and thyroids atrophied and the grafts contained primarily chromophobes. Recent work by Desclin and Flament-Durand (1969) has shown that reserpine stimulated the appearance of numerous prolactin cells in rats with pituitary homotransplants. These results were similar to those found in in situ anterior pituitary during lactation. Reserpine stimulation was absent when grafts were outside the hypophysiotrophic area.

Several workers have transplanted hypothalamic tissue along with anterior pituitary tissue. Moszkowska (1959) homografted pituitaries of male rats directly on the ovary of nonhypophysectomized, prepubertal rats. Others received transplants of pituitary plus hypothalamic tissue in close contact with each other. In the 30 rats with pituitary grafts, follicular growth was stimulated and vaginal opening occurred within 5 days after transplantation. These animals failed to develop corpora lutea. Five of 14 rats which had both hypothalamic and pituitary grafts present formed corpora lutea. To stimulate ovulation and corpus luteum formation it was necessary for hypothalamic tissue to be

present with the pituitary graft. This same procedure was used by Moszkowska and Kordon (1960). Immature nonhypophysectomized, female rats received either two female hypothalami or two male hypothalami along with the male pituitary gland. Greater ovarian stimulation was observed in the group which received the female hypothalami. Seventeen of 22 females grafted with the male pituitary and two female hypothalami formed corpora lutea but only 5 of 14 which received both male pituitary glands and male hypothalami formed corpora lutea.

The subcutaneous tissue of the flank was used by Montemurro and Gardner (1961 and 1963) as a transplantation site for pituitary and hypothalamic homografts in nonhypophysectomized, female mice. When pituitaries were homografted alone the typical hypersecretion of prolactin (luteotropin) resulted as manifested by prolonged diestrous intervals. When pituitaries were homografted in combination with hypothalamic tissue most of the pituitary tissue was fragmented and resorbed. When pituitary grafts were previously established the hypothalamic transplant had no effect on luteotropic secretion. In the previously discussed work by Halberg *et al.* (1959) it was noted that when hypothalamic tissue was transplanted in combination with pituitary gland, the carcinogenic effect of the ectopic gland was delayed. The ovulatory response to pregnant mare serum gonadotropin

(PMS) has been studied in immature, nonhypophysectomized rats bearing adenohypophysial and hypothalamic median eminence tissue homografted beneath the kidney capsule (Hopkins et al., 1968). PMS was injected on the 4th day after transplantation. Ovulation rate was depressed in rats which received an anterior pituitary graft alone. This depression was augmented when proestrous hypothalamic tissue was transplanted with the adenohypophysial tissue. Exogenous prolactin in nongrafted rats caused the same depressing effect on ovulation. They concluded that the hypothalamic graft caused the pituitary graft to produce an antagonistic substance to the gonadotropin.

The hypothalamic control of adenohypophysial function has been demonstrated by infusion of median eminence extracts (MEE) into hypophysectomized rats bearing ectopic pituitary tissue. Pituitaries were autotransplanted beneath the kidney capsule (Evans and Nikitovitch-Winer, 1965 and Zanisi et al., 1967). Intrarenal infusion of MEE for 5 to 31 days stimulated functional and cytological reactivation of the pituitary autografts in these rats (Evans and Nikitovitch-Winer, 1965). Vaginal estrus was observed in 4 of 11 animals. The ovaries contained numerous large follicles and the adrenals and thyroids were stimulated. Although vaginal estrus did not occur, the adrenals, thyroids, ovaries, and grafted pituitary tissue showed definite structural response in

five additional animals. Intraperitoneal injections of MEE in prepubertal animals for 14 days hastened the onset of puberty and produced similar stimulation of hypophysial target organs. The effect of topical administration of MEE upon pituitary grafts placed in a pneunodermal pouch under the dorsal skin of hypophysectomized rats has been studied by Ducommun and Guillemin (1966). The staining characteristics of the various chromophils were maintained, but the influence of the graft diminished as the duration of treatment increased.

Work by Piacsek and Meites (1966 and 1967) has shown that the hypothalamus influences secretion from ectopic pituitary tissue. Ovaries and uteri of hypophysectomized rats which received two subcutaneous pituitary homotransplants and were exposed to continuous light for 21 days were significantly heavier than similar rats exposed to 14 hours of light daily. These increases were due to increased follicular development. This effect could be augmented by HCG treatment for 3 to 5 days before autopsy. Exposure of hypophysectomized rats without pituitary transplants to constant light failed to produce ovarian or uterine weight increases. The transplanted pituitaries in rats exposed to constant light were larger, possessed many more viable cells and contained more cellular stain than the pituitary transplants of rats exposed to controlled light. Piacsek et al. (1969) found that exposure to constant light caused an

increase in FSH concentration of approximately 125% in the pituitary transplants. These influences of constant light were inhibited by placement of lesions in the basal medial hypothalamus. Beddow and McCann (1969) have used bilateral median eminence lesions to demonstrate the hypothalamic influence upon ectopic pituitary tissue. Weanling hypophysectomized male rats received ten intramuscular pituitary homotransplants. Growth and testicular and accessory gland development were intermediate between hypophysectomized and intact rats. Unilateral castration of grafted rats caused compensatory hypertrophy of the remaining testicle. When the median eminence was lesioned bilaterally 40 days after pituitary transplantation, there was an inhibition of growth and regression of testes and accessory glands. Testicular compensatory hypertrophy could not be induced in these animals.

Morphology of Pituitary Transplants

In the majority of work on pituitary transplantation the condition of the grafts usually was described briefly. From the many reports already discussed the adenohypophysial portion of grafts implanted in sites remote from the hypothalamus contained large areas of central necrosis and varying degrees of connective tissue infiltration. The neurohypophysial portion completely degenerated. The remaining pars distalis tissue contained mostly chromophobes and

acidophils.

In 1939, Phelps et al. gave thorough descriptions of anterior pituitary grafts inserted in a thigh muscle of female rats for 5, 10, 20 or 60 days. After 5 days the central area of the anterior lobe showed signs of degeneration such as pyknotic nuclei and indistinguishable cell boundaries. There was a narrow border of normal tissue on the periphery. As time progressed the central area was replaced with a loose network of connective tissue and later a small core of dense connective tissue which radiated to the periphery. By 20 to 60 days later the grafts contained primarily chromophobes with significant numbers of acidophils. Mitotic figures were also observed.

Wolfe et al. (1940) described the condition of anterior pituitary homografts 7 to 10 months after transplanting them near the mammary glands of nonhypophysectomized, female mice. The transplants were surrounded by a fibrous capsule from which connective tissue grew into the graft. Most grafts contained small, closely packed chromophobes. In some cells the boundaries were visible, but in others they were indefinite. Basophils were not observed, but a few acidophils were seen. It has been observed in several strains of mice that after prolonged periods of transplantation, significant numbers of animals bearing subcutaneous pituitary grafts (Gardner, 1960) or intraocular pituitary grafts

(Bardin and Liebelt, 1960) developed large tumors in the ectopic pituitary tissue. Bardin and Liebelt (1960) described them as chromophobe adenomas. Siperstein and Greer (1956) transplanted pituitaries of newborn mice to the anterior chamber of the eye in adult female mice and recovered grafts from 1 day to 14 months later. Histological examination revealed that during the first 1 to 8 days the anterior pituitaries were in various stages of degeneration. There was marked stasis of blood and engorgement of the sinusoids. Large areas of necrotic tissue were often observed in a central cavity. The anterior lobe tissue regenerated after the first week, but cytologic staining reactions were less than observed in adenohypophysial tissue of control animals. Acidophils were rarely observed by the 51st day after transplantation. Only a few basophils were observed in a minority of grafts in the 15 to 36 day period. A large proportion of cells was chromophobes which were larger than normal and had characteristics indicative of cellular activity. In later time periods the chromophobes formed palisade patterns. The pars tuberalis and the pars intermedia survived, but the posterior lobe could not be identified by 15 days after transplantation.

In 1959, Martini et al. described histological changes in rat anterior pituitary tissue transplanted to the anterior chamber of the eye of male rats, which were hypo-

physectomized 2 to 3 days after transplantation. After several months the grafts were encapsulated with variable degrees of infiltration of connective tissue. A massive central infarct was not found. Chromophobes with vesicular nuclei made up most of the cellular constituents. Some beta basophils were observed, but gonadotrop basophils were rare or absent. Kovacs (1961) found the development of a massive central infarct in homografts of rat pituitaries transplanted to the anterior chamber of the eye of nonhypophysectomized rats. Almost all chromophils became degranulated during the first or second week after transplantation. After 6 weeks only very rare shrunken basophils and a few acidophils could be recognized. The cytology of pituitary autotransplants in adult female and prepubertal rats of both sexes was studied by Sanders and Rennels (1957). After 2 to 4 months beneath the kidney capsule the grafts were composed of chromophobes and prolactin-type acidophils. Thyrotropes and gonadotropes were absent.

An electron microscope study of pituitary tissue autografted beneath the kidney capsule of adult estrous rats was made by Rennels in 1962. Grafts were removed 18 days after transplantation. All cell types were observed, but the predominant cell was acidophilic. These cells contained an eccentric nucleus, large polymorphic secretory granules with immature granules concentrated in the Golgi area and

elaborate arrays of endoplasmic membranes with associated ribonucleoprotein particles.

MATERIALS AND METHODS

The maintenance of corpora lutea was investigated in immature pigs in which the adenohypophysis was autotransplanted to a site between muscle bundles of the m. gracilis. Control animals consisted of immature hypophysectomized pigs in which a sham operation was performed at the m. gracilis. After adenohypophysial autotransplantation or hypophysectomy, ovulation was induced 28 to 90 days later by injecting intramuscularly desiccated porcine adenohypophysis and human chorionic gonadotropin (HCG). These gonadotropins stimulated follicular development, ovulation, and formation of corpora lutea. The day of ovulation was designated day 1.

To determine luteolytic action by the uterus, pigs with either an adenohypophysial autotransplant or hypophysectomy were then hysterectomized at days 8 to 10. The experimental groups were as follows: (a) adenohypophysial autotransplantation and hysterectomy (T - U), (b) adenohypophysial autotransplantation and sham hysterectomy (T + U), and (c) hypophysectomy and hysterectomy (H - U). After induction of ovulation the pigs were sacrificed at day 25, with the exception of two animals which were killed at day 35. Ovarian function, particularly luteal function, was evaluated by histological examination and by determination of progesterone in the ovary.

Surgical Methods

Hypophysectomy

The experimental pigs were maintained in a constant environment at least 3 days before surgery. Within 1 hour of surgery 12.5 to 25 mg of cortisone acetate (Cortone Acetate; Merck, Sharp and Dohme) was injected intramuscularly. Endotracheal intubation was performed under light anesthesia which was induced by intravenous injections of approximately 0.5 g thiopental sodium (Abbott Laboratories). Pigs were then maintained under surgical anesthesia on a closed-circuit system of halothane (3 to 5%) and oxygen (400 to 600 ml/min) for the duration of the hypophysectomy and the pituitary transplantation or sham transplantation, which required approximately 3 hours. Hypophysectomy was performed by the supra-orbital approach as described by du Mesnil du Buisson et al. (1964).

Immediately after removing the pituitary gland from the sella turcica in those animals which were to receive an adenohypophysial autotransplant, the anterior lobe was sliced into pieces 1 to 2 mm thickness. While closing the wound for the hypophysectomy and preparing the transplantation site, these slices were maintained in 20 to 30 ml of tissue culture medium (TC-199; Difco Laboratories) under 95% O_2 + 5% CO_2 at 37° C in a shaking metabolic incubator (Dubnoff). The adenohypophysis was incubated 20 to 30

minutes.

In the pigs receiving an adenohypophysial autotransplant, a small incision was made in the fascia of the m. gracilis. A tunnel was formed between muscle fiber bundles by blunt dissection. Thin slices of adenohypophysial tissue were evenly distributed within this tunnel and the fascia was closed with 4-0 nylon.

The animals were given an intravenous infusion of 1,000 ml of normal electrolytes containing 5% glucose and an intramuscular injection of 12.5 to 25 mg of cortisone acetate at the conclusion of surgery. Feed and water were given by gavage twice daily until the animals could eat unassisted. They ate and drank unassisted within 1 to 3 days. The pigs were maintained in a controlled environment (21° C) after surgery.

Laparotomy

For hysterectomy or sham hysterectomy the animal was maintained under halothane and oxygen anesthesia and laparotomized midventrally at days 8 to 10. The corpora lutea were marked with loops of 4-0 nylon in all experimental animals. Hysterectomies or sham hysterectomies were performed on pigs which were previously subjected to adenohypophysial autotransplantation or hypophysectomy.

Induction of Ovulation

Three to 4 weeks after hypophysectomy or autotransplantation of the anterior pituitary gland the pigs were given intramuscular injections of 25 mg of desiccated porcine anterior pituitary twice daily in 2 ml of physiological saline. In pigs which responded to this initial injection level of pituitary powder, the vulva became edematous and pink within 5 to 7 days, indicating that maturation of ovarian follicles had occurred. Beginning on the third day of the vulvular response, two daily injections of 1,000 IU HCG were given intramuscularly or intravenously in addition to the desiccated anterior pituitary to insure ovulation. Exogenous hormone treatment was stopped at this time. The day after the second injection of HCG was designated as the day of ovulation or day 1. If animals did not respond to the first series of gonadotropic hormone injections, the treatment regimen was repeated with a higher dosage of desiccated anterior pituitary after a period of 1 to 2 weeks.

Potency of the porcine adenohypophysial tissue was equivalent to 101.8 μg NIH-FSH-S_I/mg of dried tissue as determined by the HCG augmentation method of Steelman and Pohley and 28.9 μg NIH-LH-S_I/mg of dry powder as determined by the ventral prostate weight assay.

Histology

Luteal, ovarian, and uterine tissues recovered between days 8 and 10 and at day 25 were fixed in 10% formalin, sectioned at 10 μ and stained with hematoxylin-eosin. Sella turcicas along with the basal area of the hypothalamus from pigs in which the pituitary gland was removed in more than one piece were fixed in 10% formalin. The sphenoid bone was dissected from the dura-mater of the sella turcica before embedding in paraffin. These sella turcicas were sectioned serially at 10 μ, stained with hematoxylin-eosin and examined for remnants of adenohypophysial tissue. The sella turcicas of pigs in which the pituitary gland was removed without fragmentation were not examined histologically.

A section of the muscle containing the autotransplant of the adenohypophysis was fixed in Susa's fixative and sectioned at 5 μ. The sections were stained with aldehyde-thionine-PAS and counter stained with orange-G as described by Ezrin and Murray (1963) and Anderson et al. (1967).

Progesterone Analysis

Duplicate samples of 210 to 916 mg of luteal tissue were collected between days 8 and 10. When the animals were sacrificed at day 25 or 35, duplicate samples of approximately one-half of an ovary were prepared for determination of total ovarian progesterone content.

Tissues were stored in 60 ml of 95% ethanol at -20° C

until they were analyzed. The procedures for progesterone analysis were similar to those reported by Duncan et al. (1960) and Masuda et al. (1967). At the time of tissue homogenization progesterone-^{3}H was added for later determination of the percentage of progesterone recovery. The homogenized tissues were extracted three times with 60 ml 95% ethanol at 75° C for 50 minutes. The ethanol was evaporated and the residue was redissolved in n-hexane and stored overnight at 5° C to aid precipitation of insoluble lipids. Adsorption chromatography was performed on aluminum oxide gel columns using chloroform-n-hexane (95:5) as the eluent. This fraction was evaporated, transferred quantitatively to a centrifuge tube with n-hexane, dried under nitrogen, and redissolved in 200 µl benzene-dichloromethane (1:1) for application to a thin layer chromatography plate of silica gel G. The sample was chromatographed in n-hexane-ethyl acetate (5:2) and chromatographed in a second dimension in dichloromethane-ether (5:2). The area containing the progesterone was observed under ultra-violet light and scraped off the plate. After eluting with 10 ml 95% ethanol, an aliquot was removed to determine percent recovery by liquid scintillation counting (Packard Tri-Carb, Model 3008) and progesterone was determined by UV absorption at 240 mµ (Beckman DU).

For identification of the isolated steroid, individual

samples of progesterone were pooled and dissolved in chloroform. Infrared absorption spectra (Beckman IR-12) were determined on the isolated steroid and authentic progesterone. Infrared absorption spectra of the steroid from ovarian tissue indicated that progesterone was the substance isolated.

RESULTS AND DISCUSSION

Survival Rates of Pigs Following Hypophysectomy

The survival rate of hypophysectomized pigs is low over prolonged periods due to several factors such as the trauma of hypophysectomy itself, the inability to adapt to minor stressful conditions, and the occurrence of electrolyte imbalance. Diabetes insipidus manifested by polyuria is probably the major factor contributing to the occurrence of electrolyte imbalance.

Seventy-two crossbred female pigs, 3 to 5 months of age and 24 to 77 kg body weight were hypophysectomized. The appendix presents the treatment, age and weight at hypophysectomy and the survival period of each animal. Ten animals (14%) died at hypophysectomy. Most of these animals showed signs of porcine stress syndrome. This syndrome progressed as follows: irregular heart beat, cessation of respiration, tetany of the entire body, body temperature increase to above 43° C and cardiac arrest. Seventeen animals (24%) died within 5 days after hypophysectomy. These animals showed muscular weakness, especially in the front legs, and a lack of desire to eat. Despite intraperitoneal infusions of normal electrolytes containing 5% glucose, these pigs eventually died. Body temperature was below normal for several hours preceding death. Twenty-nine pigs (40%) died because of electrolyte imbalance. The earliest

sign of this condition was uncoordinated movements such as stumbling while walking. As this condition progressed, painful convulsions occurred and eye movements were erratic. Within several hours of death the heart rate was above 200 per minute and clear watery fluid flowed from the nostrils. If normal electrolytes were infused early enough these animals recovered. Electrolyte imbalance usually did not occur until after 2 weeks following surgery. Three (4%) pigs died during laparotomy for hysterectomy or sham hysterectomy due to the stress of anesthesia and surgery. The remaining pigs (18%) were assigned to the experimental treatments.

Ovarian Function After Induction of Ovulation

Twenty-seven animals survived after hypophysectomy, autotransplantation of the adenohypophysis and hysterectomy until various periods following the administration of desiccated anterior pituitary tissue. Sixteen of 21 adenohypophysial autografted animals and four of six hypophysectomized animals ovulated. To induce ovulation one to four series of injections of desiccated porcine pituitary at 25 to 75 mg two times per day were required. Table 1 presents the ovarian morphology 8 to 10 days after ovulation. Extensive follicular growth occurred, resulting in the presence of follicles 4 to 20 mm in diameter. Corpora lutea were hyperemic and attained a diameter of 6 to 10 mm which was

Table 1. Ovarian morphology of pigs 8 to 10 days after induced ovulation

Experimental group	Left ovary		Right ovary		Total CL
	Follicles	CL[a]	Follicles	CL	
Adenohypophysial autotransplantation					
4225			7	2	2
4653	24	6	24	10	16
4652	18	11	20	4	15
4692	5	13	18	9	22
5700	15	10	12	12	22
9021	20	3	18	3	6
9090	12	9	12	14	23
9134	2	0	0	2	2
9301	0	4	0	4	8
9312	1	7	5	5	12
9361	2	1	2	0	1
9355	15	6	12	11	17
9370	6	5	4	6	11
9433	10	5	12	1	6
9736	0	8	24	7	15
6110	3	6	0	8	14

[a]Corpora lutea.

Table 1. (Continued)

Experimental group	Left ovary		Right ovary		Total CL
	Follicles	CL[a]	Follicles	CL	
Hypophysectomy					
4343	2	4	1	2	6
6063	10	12	15	13	25
6404	4	0	3	1	1
6502	12	15	10	17	32

similar to that described by Corner (1921) for this stage of the luteal phase in intact sows. Luteal morphology was similar to that described by Corner (1919). Large cells with abundant cytoplasm and large nuclei with prominent nucleoli were present (Figure 1). Analysis of luteal progesterone presented in Table 2 demonstrated that the induced corpora lutea were functional. Progesterone levels were similar to those reported by Masuda et al. (1967) for day 8 of the estrous cycle.

Eleven of the 20 animals which ovulated survived the complete experimental period. Table 3 presents the number of animals completed in each treatment group. In two of the six animals in group T - U, one ovary was recovered at day 25 and the other at day 35. Corpora lutea were maintained

Figure 1. Luteal tissue 8 to 10 days after induction of ovulation (560x). Luteal cells contain abundant cytoplasm with large nuclei and prominent, dark staining nucleoli

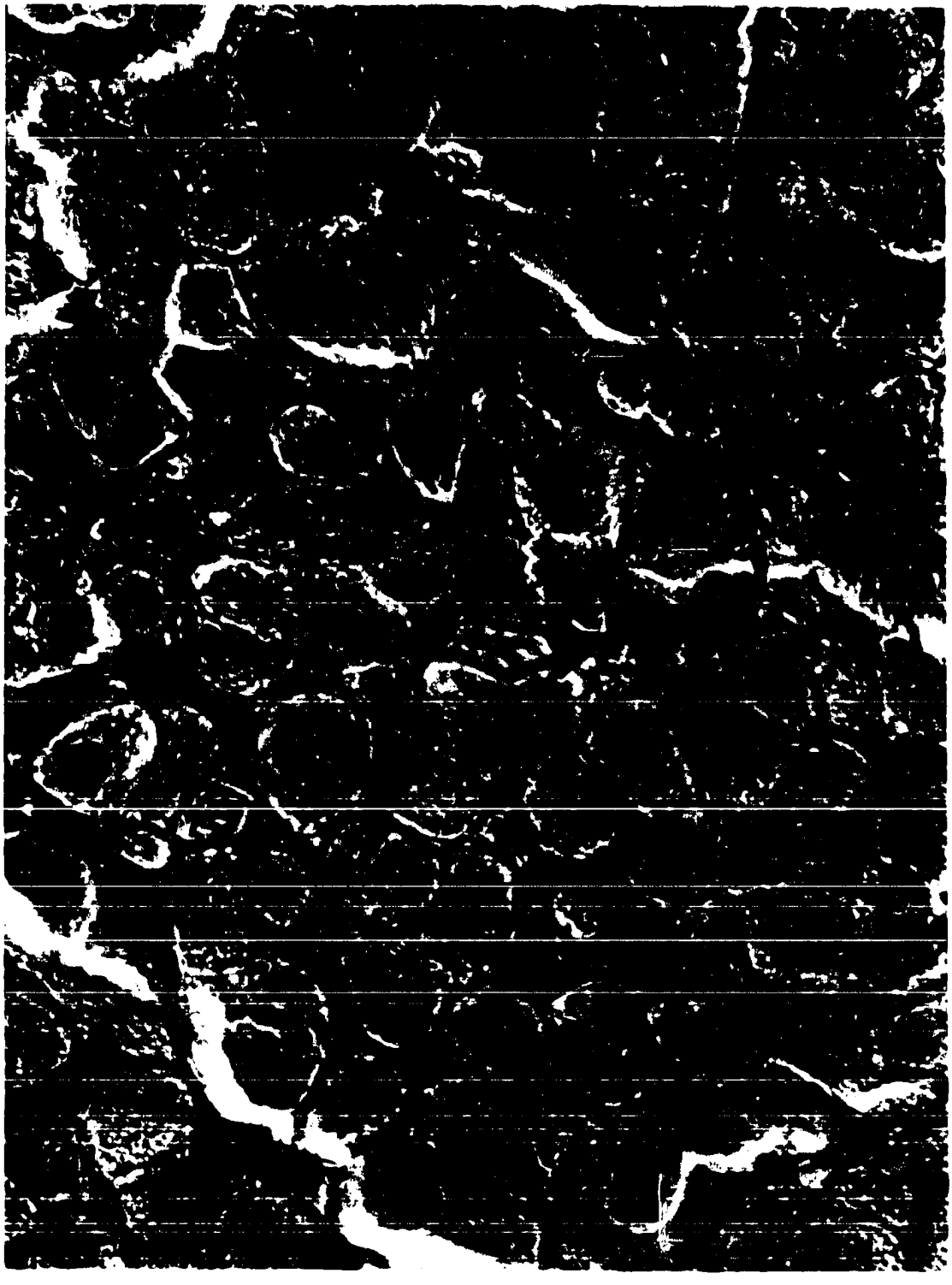


Table 2. Progesterone concentrations in luteal tissue 8 to 10 days after induced ovulation in the pig

Pigs	Sample	µg/g	Ave. µg/g
4692	1	54.8	65.6
	2	76.3	
9090	1	20.4	
	2	Nondetectable	
9312	1	55.3	53.6
	2	51.8	
5700	1	36.0	34.4
	2	32.7	
6502	1	Nondetectable	
	2	Nondetectable	
9355	1	47.0	63.0
	2	78.9	
9736	1	40.9	64.2
	2	87.4	
6110	1	87.8	91.5
	2	95.3	
6063	1	57.6	
	2	Poor recovery	
9433	1	92.5	67.2
	2	42.0	

Table 3. Number of pigs which completed the experiment

T - U[a]	T + U[b]	H - U[c]
6	3	2

[a]Adenohypophysial autotransplant and hysterectomy.

[b]Adenohypophysial autotransplant, intact.

[c]Hypophysectomy and hysterectomy.

in all animals in this group including those at day 35. Corpora lutea were reduced to 3 to 5 mm diameter, but histological examination showed that the luteal cells were in similar condition to those recovered at day 8 to 10 (Figure 2). These small corpora lutea were well vascularized and contained large luteal cells with abundant cytoplasm and large nuclei with prominent nucleoli. Analysis of total ovarian progesterone presented in Table 4 demonstrated that these maintained corpora lutea were functional. Histological examination of the interstitium of the ovary at day 25 and 35 showed atrophied interstitial tissue with reduced vascularity indicating an absence of LH stimulation. Tertiary follicles at various stages of development up to the formation of the cumulus oophorus were present, but graffian follicles were absent. This follicular development was

Figure 2. Luteal tissue 25 days after induction of ovulation and hysterectomy in a pig bearing an adenohypophysial autotransplant (560x). Large amounts of cytoplasm and prominent nuclei with dark staining nucleoli show that the luteal cells were maintained similar to those of pituitary intact pigs

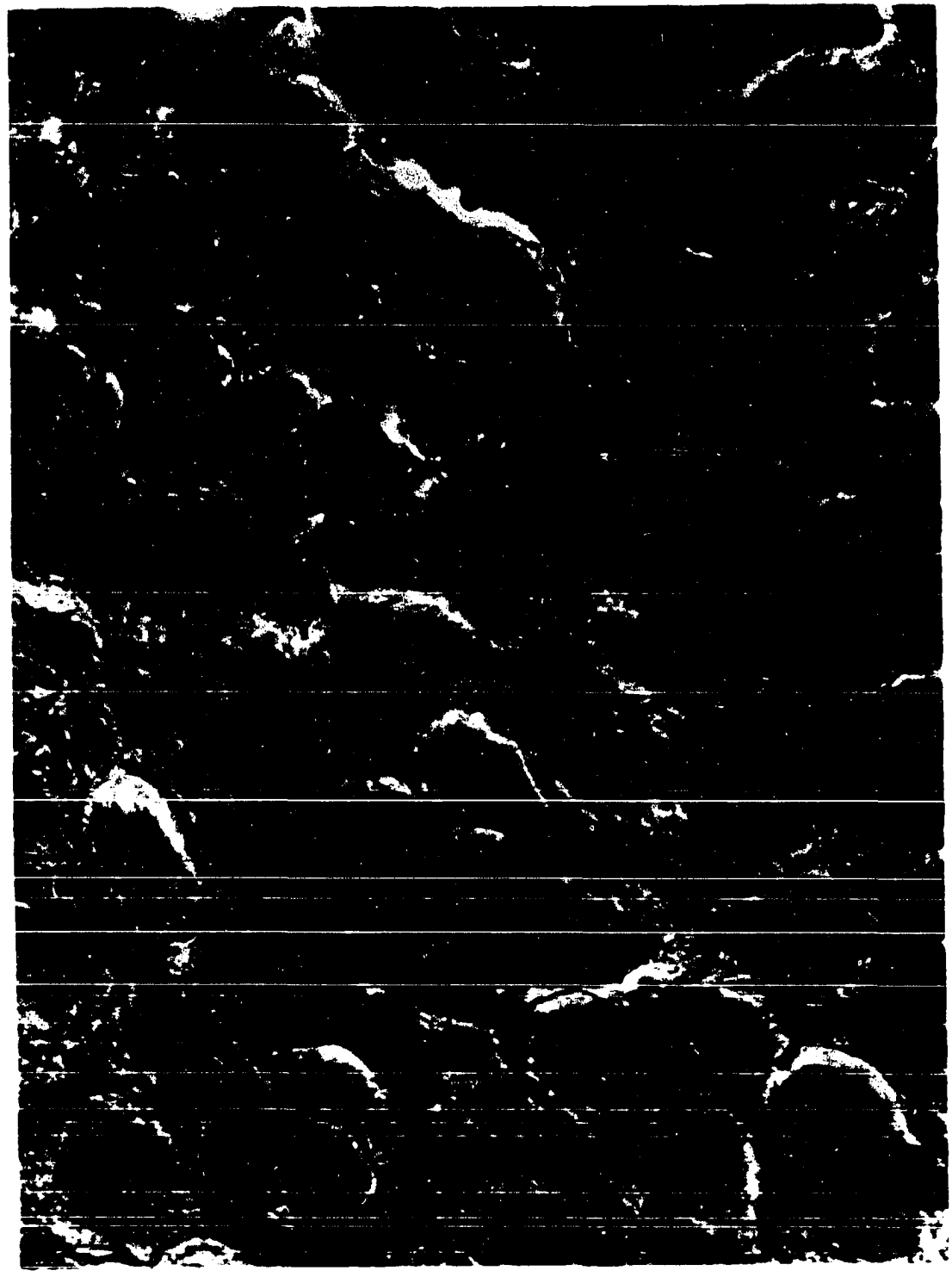


Table 4. Total ovarian progesterone content of one ovary 25 and 35 days after ovulation

Group	25 days after ovulation				35 days after ovulation			
	Sample	µg/g	Ave. µg/g	Total	Sample	µg/g	Ave. µg/g	Total
T - U								
9090	1	18.8	17.2	60.9	1	26.0	20.5	57.4
	2	15.5			2	15.0		
9312	1	Nondetectable						
	2	8.8						
4653	1	16.7	10.4	18.9				
	2	4.2						
6110	1	6.6	5.8	19.0				
	2	5.1						
T + U								
9355	1	Nondetectable						
	2	Nondetectable						
9736	1	Nondetectable						
	2	Nondetectable						
H - U								
6502	1	Nondetectable						
	2	Nondetectable						
6063	1	10.0	11.6	83.0				
	2	13.2						

similar to that of hypophysectomized nongrafted animals, suggesting that FSH activity was absent. Figure 3 demonstrates the ovarian histology described for group T - U at 25 or 35 days after ovulation.

In group T + U three animals survived the complete experimental period of 25 days after ovulation, and one animal died on day 15. In all cases corpora lutea present at day 8 to 10 had completely regressed to typical corpora albicantia (Figure 4). Histology of the interstitial tissue and follicular apparatus was similar to that of the T - U group (Figure 5). Progesterone analysis presented in Table 4 demonstrated the absence of luteal function in the T + U animals at day 25. The luteolytic influence of the uterus in pituitary intact pigs has been extensively reviewed by Melampy and Anderson (1968). Mature pigs possessing a nongravid uterus show estrous cycles of 18 to 23 days. Hysterectomy during the luteal phase of the estrous cycle results in maintenance of corpora lutea for approximately the length of normal gestation (112 days). The results of the T + U group indicate that the uterine luteolytic mechanism acts either through the pituitary gland itself or directly on the corpora lutea. The central nervous system is probably not involved in this mechanism.

Only two animals survived in the H - U group. A third animal from the T - U group which died on day 17, should be

Figure 3. Ovarian tissue 25 days after induction of ovulation and hysterectomy in a pig bearing an adenohypophysial autotransplant (11x). The interstitial tissue is atrophied and there is an absence of follicular development. Small but morphologically normal corpora lutea are present

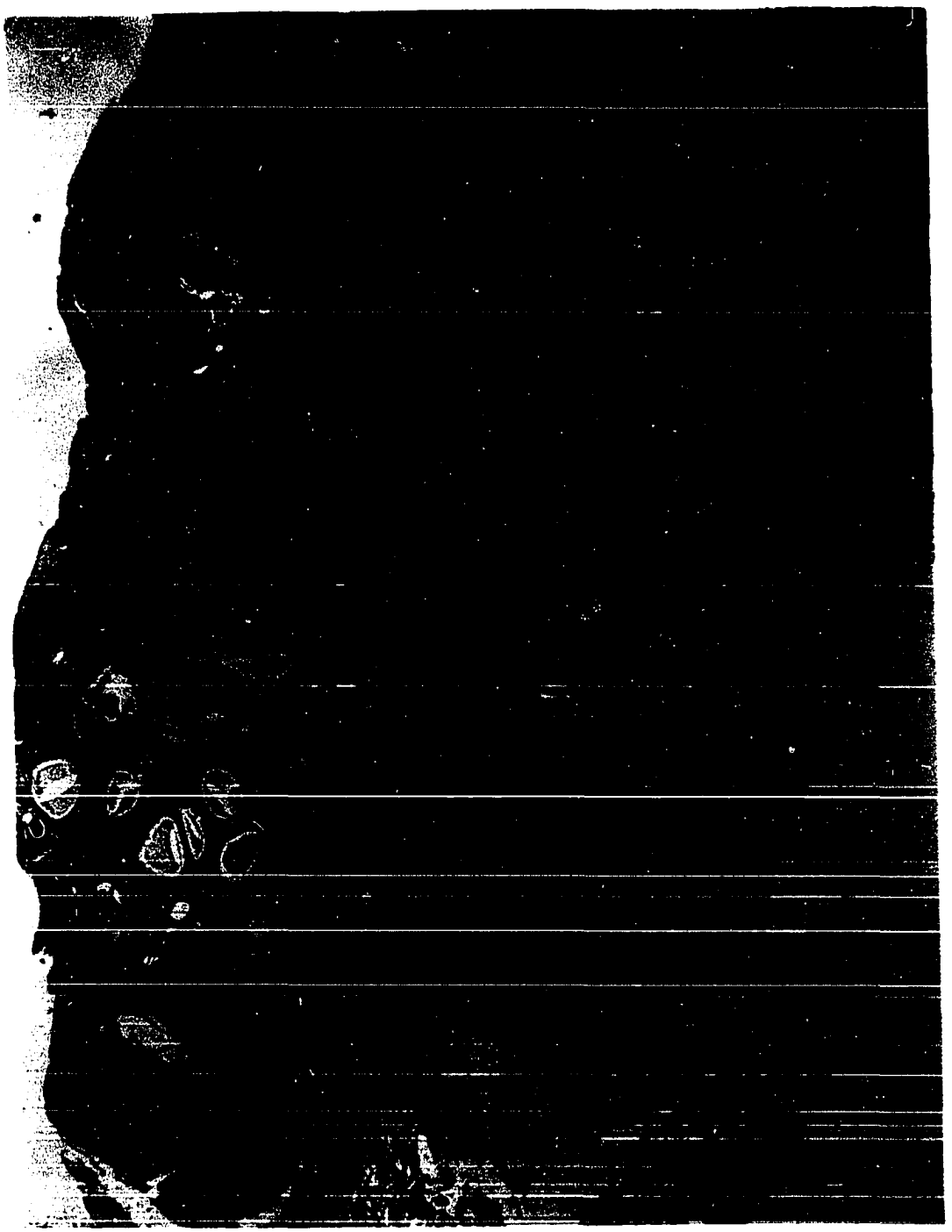


Figure 4. Corpus albicans 25 days after induction of ovulation and sham hysterectomy in a pig bearing an adenohypophysial autotransplant (34X)

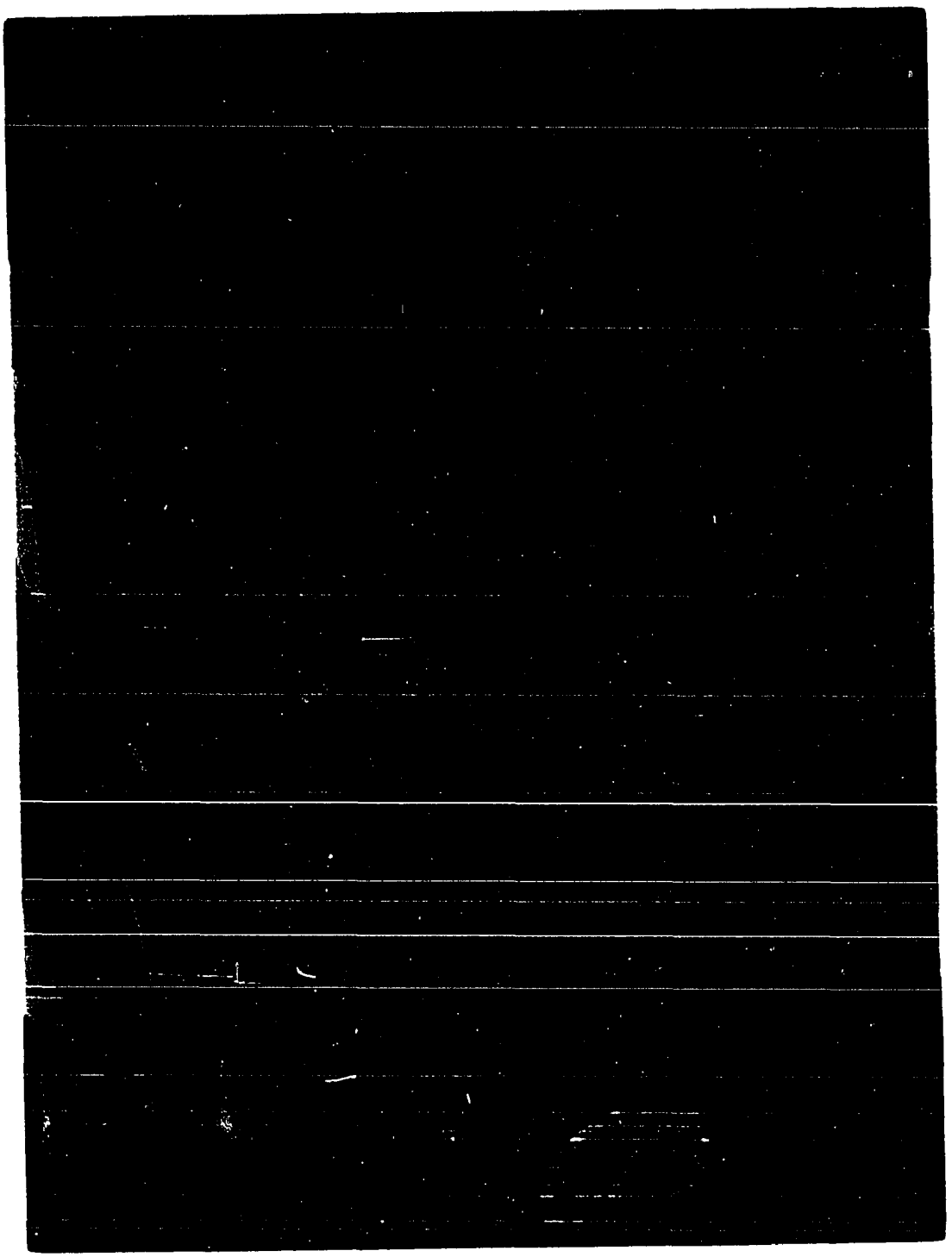
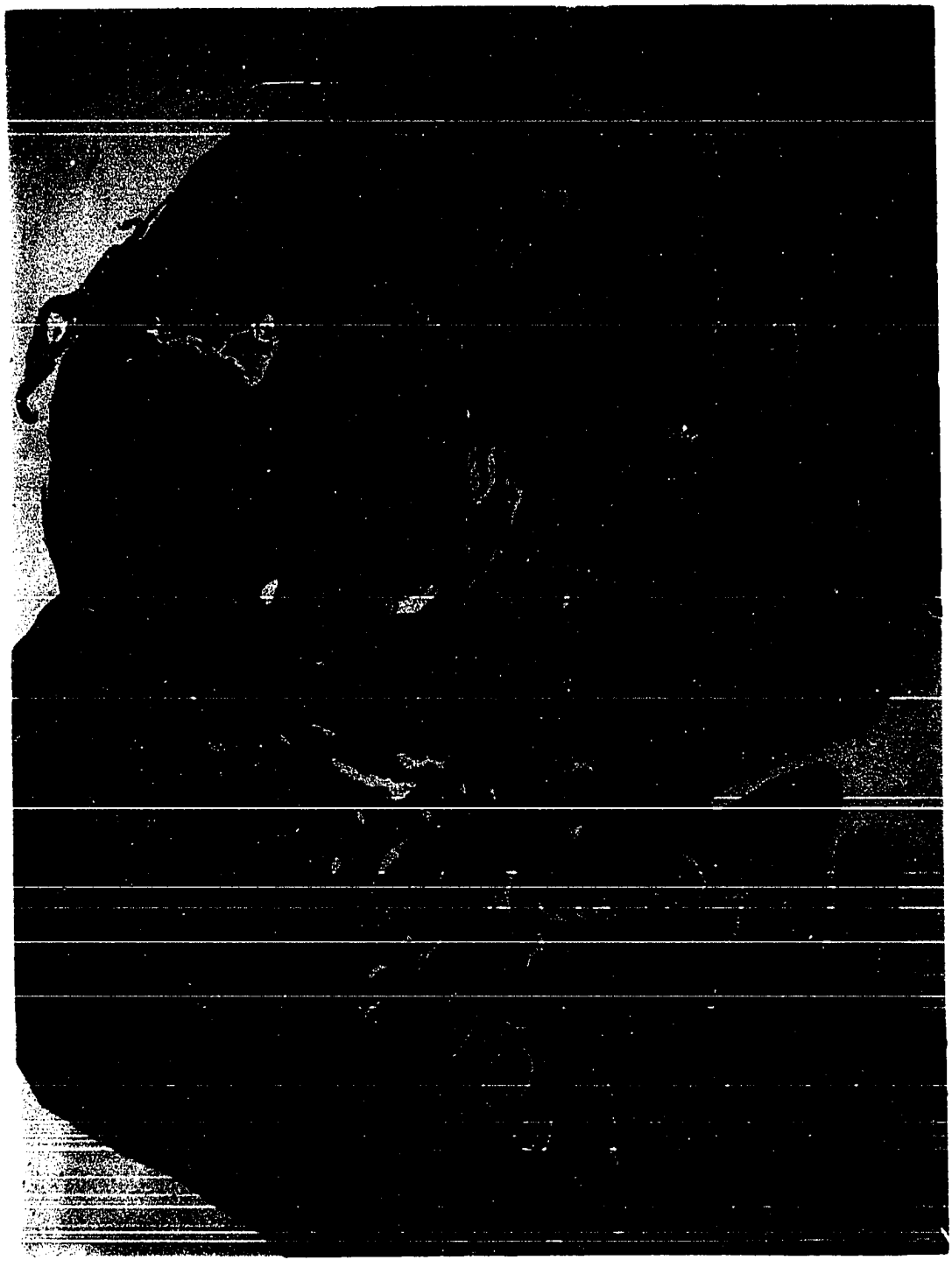


Figure 5. Ovarian tissue 25 days after induction of ovulation and sham hysterectomy in a pig bearing an adenohypophysial autotransplant (11x). The interstitial tissue is atrophied and there is an absence of follicular development. Corpora lutea from induction of ovulation have regressed to corpora albicantia



considered when evaluating the H - U group. No pituitary tissue was found at death and the corpora lutea were in various stages of regression. Corpora lutea of one H - U animal which survived the complete experimental period were completely regressed; however, the other animal possessed corpora lutea which were similar to those of animals in group T - U. The condition of the interstitial tissue and follicular apparatus of group H - U was similar to animals in groups T - U and T + U (Figures 3 and 5). Analysis of total ovarian progesterone (Table 4) in the H - U animal with completely regressed corpora lutea shows an absence of luteal function in this animal.

The results from group T - U indicate that the adenohypophysial grafts are secreting a luteotropic substance(s) but the unexpected maintenance of luteal tissue in one animal from group H - U demonstrates the necessity for more adequate control animals before definite conclusions may be drawn. It must also be considered that the anterior pituitary grafts failed to secrete luteotropin and that the inherent life span of induced corpora lutea may be greater than those formed at spontaneous ovulation. The condition of the follicles and interstitial tissue of day 25 ovaries from graft bearing animals suggest that the adenohypophysial grafts did not secrete LH or FSH.

If the anterior pituitary grafts produced luteotropin

then several explanations for the decreased size of the maintained corpora lutea should be considered. The decreased vascularity of the atrophied ovarian interstitial tissue may have influenced the quantity of maintained luteal tissue in the T - U pigs. Maintenance of the corpus luteum in the pig might be controlled by a luteotropic complex similar to that of FSH and LtH in the hamster (Turnbull and Kent, 1966; Greenwald, 1967 and Choudary and Greenwald, 1967); therefore, if all components of this suggested luteotropic complex were present then luteal size might have been maintained. The amount and condition of the surviving adenohypophysial tissue in the autotransplants probably influenced the quantity of substance(s) secreted by the grafts, but several workers have obtained abundant secretion of luteotropin in the rat from adenohypophysial grafts which contained a thin shell of viable tissue surrounding a large central infarct.

Corpora lutea were maintained as long as 35 days in adenohypophysial autografted pigs which were hysterectomized 8 to 10 days after induction of ovulation; whereas, the corpora lutea regressed within 25 days in intact, autografted animals. These results indicate that the luteolytic mechanism of the uterus acts either through the adenohypophysis itself or directly on the corpora lutea. If further work in this laboratory on ovarian function after induced ovula-

tion in hypophysectomized pigs confirms the suggested luteotropic influence of ectopic adenohypophysial tissue in the pig, this investigation indicates that the luteotropin of the pig is controlled by an inhibiting influence of the central nervous system.

Morphology of the Adenohypophysial Autotransplants

The quantity of adenohypophysial tissue recovered was approximately 10 to 20% of the original tissue transplanted. This is a crude estimation based on general observations. The condition of adenohypophysial tissue varied between animals and between areas within the same pig. Figure 6 presents photomicrographs of anterior pituitary autotransplants illustrating the variable condition of the grafts. Acidophils and chromophobes were the only adenohypophysial cells found except for the rare occurrence of a few basophils. The morphology of the anterior pituitary grafts in this study is similar to that found by many workers for adenohypophysial grafts in rats, mice, hamsters and guinea pigs.

Figure 6. Adenohypophysial autotransplants in the pig. Examples of well developed adenohypophysial grafts are shown in photomicrographs A, B, and C (560x)

A. The sinusoid reticulum demarcates acinar formations of adenohypophysial cells similar to intact adenohypophysial tissue. Orange-brown acidophils with large amounts of granulated cytoplasm are present in an acinar formation in the center. A purple basophil is to the left of these acidophils. A basophil is also present at the medial right edge of the photomicrograph. Basophils were rarely present. Orange-brown acidophils are also dispersed throughout the graft. Light staining cells, which may be chromophobes, are present in groups within the acinar formations

B. Yellow-orange acidophils with abundant granulated cytoplasm indicative of functional cells are present in acinar formations. Cells which appear to be chromophobes are in the lower right portion of the photomicrograph

C. Acinar formations similar to intact adenohypophysis are not present and the sinusoid reticulum has changed to an irregular network of collagenous fibers. Yellow acidophils and cells which have no cytoplasm or light staining cytoplasm are distributed throughout the graft

Photomicrographs D, E, F, and G are examples of pituitary grafts which are well developed but are not as well organized as those in photomicrographs A, B, and C

D. Yellow cells which are similar to functional adenohypophysial acidophils are adjacent to the abnormally dense sinusoid reticulum. Cells predominate which are faintly stained pink with PAS. These cells may be chromophobes

E. Yellow acidophils with moderate amounts of cytoplasm predominate. These groups of acidophils do not possess the acinar arrangement typical of intact adenohypophysial tissue

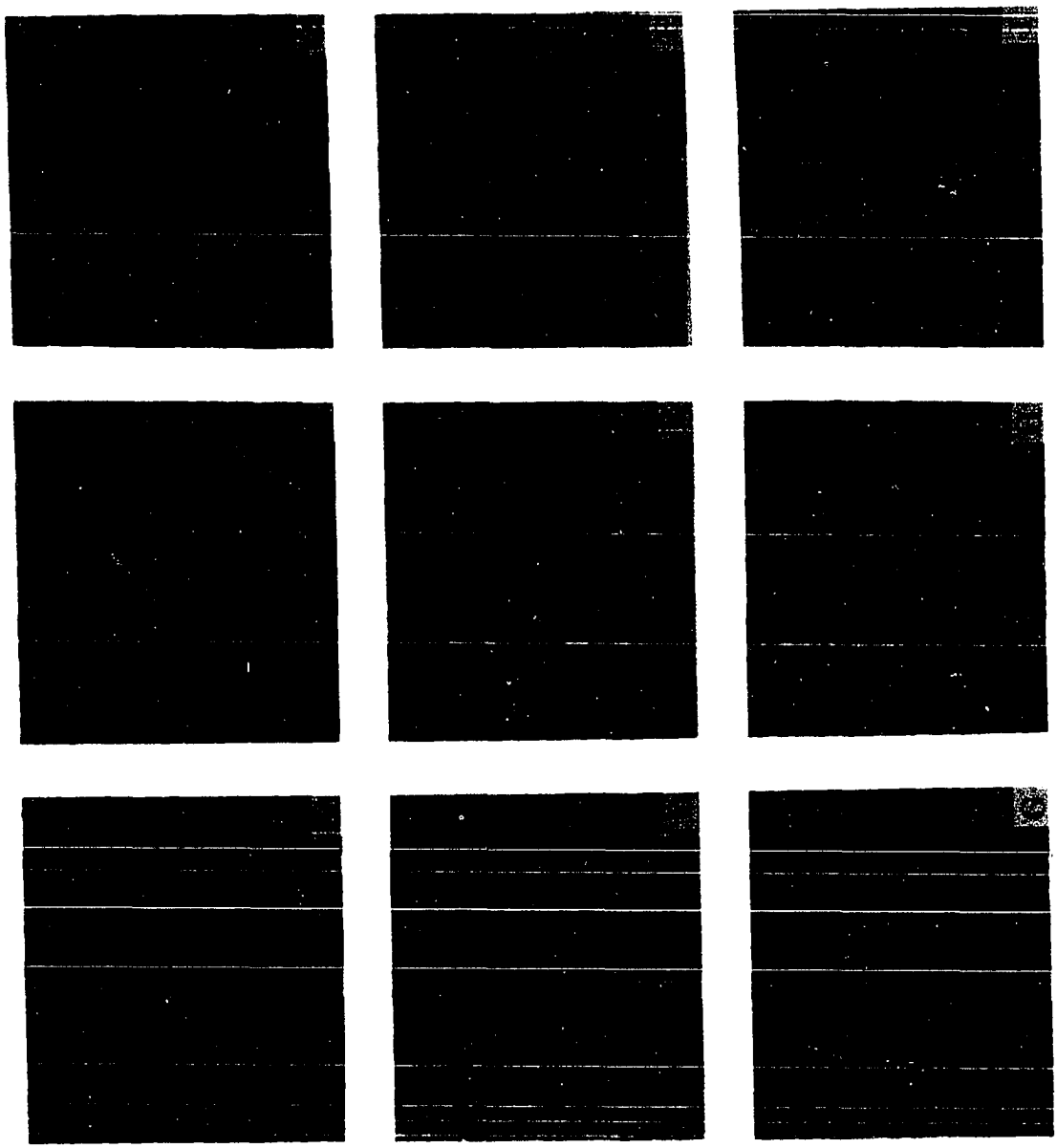


Figure 6. (Continued)

F. An abundant vascular supply is present at the top of the photomicrograph. Small adenohypophysial cells which may be classified as chromophobes are arranged in parallel cords separated by thin reticular fibers. These cells contain variable amounts of cytoplasm which did not stain

G. Small adenohypophysial cells similar to those in F are present in a disorganized array indicating degeneration of the anterior pituitary tissue. The sinusoid reticulum has degenerated to an irregular network of connective tissue fibers. However, an abundant blood supply is indicated at the left of the photomicrograph

Examples of completely degenerated anterior pituitary tissue are presented in photomicrographs H and I

H. The adenohypophysial tissue has completely degenerated and has been replaced by fibroblasts and connective tissue development

I. Fibroblasts and connective tissue fibers have replaced most of the adenohypophysial tissue

SUMMARY AND CONCLUSIONS

Ovarian function was investigated in immature female pigs after hypophysectomy or autotransplantation of the adenohypophysis to a site between muscle bundles of m. gracilis. Ovulation was induced 28 to 90 days later by intramuscular injections of desiccated porcine adenohypophysis and intramuscular or intraveneous injections of human chorionic gonadotropin. These gonadotropins stimulated follicular development, ovulation, and formation of corpora lutea. The day of ovulation was designated day 1. To determine the luteolytic action by the uterus, hypophysectomized pigs and pigs with an adenohypophysial autotransplant were hysterectomized at days 8 to 10. The experimental groups were as follows: (a) adenohypophysial autotransplantation and hysterectomy (T - U), (b) adenohypophysial autotransplantation and sham hysterectomy (T + U), and (c) hypophysectomy and hysterectomy (H - U). After induction of ovulation the pigs were sacrificed at day 25, with the exception of two animals which were killed at day 35. Ovarian function, particularly luteal function, was evaluated by histological examination and by determination of progesterone in the ovary.

Corpora lutea were hyperemic and attained a diameter of 6 to 10 mm by day 8 to 10. Large cells with abundant cytoplasm and large nuclei with prominent nucleoli were present.

These induced corpora lutea were functional as indicated by concentrations of progesterone similar to those found in intact pigs during the active luteal phase of the estrous cycle. In the T - U group corpora lutea were present at 25 and 35 days after induction of ovulation. The size of these corpora lutea were reduced to 3 to 5 mm diameter, but the cellular morphology was similar to that of corpora lutea at day 8 to 10. Progesterone analysis demonstrated that the ovaries in these experimental animals contained progesterone. In the T + U group the corpora lutea regressed completely by day 25. The interstitial tissue of the ovaries was atrophied and mature follicles were absent in all adenohypophysial autotransplanted pigs.

The corpora lutea of one pig in group H - U and one pig in group T - U, which had no adenohypophysial tissue present at the transplantation site, regressed by day 25. However, at day 25 corpora lutea of another pig from the H - U group were maintained similar to those from group T - U, indicating the necessity of more control animals before definite conclusions may be drawn.

Results from group T + U indicate that the uterine luteolytic effect acts either through the pituitary gland itself or directly on the corpora lutea. The histology of the interstitial tissue and follicular apparatus of the T - U and T + U pigs was similar to hypophysectomized con-

trols, indicating an absence of LH and FSH secretion by the ectopic pituitary tissue. Results from group T - U indicate that the ectopic pituitary tissue secreted a luteotropic substance(s). If further work in this laboratory on ovarian function after induced ovulation in hypophysectomized pigs confirms this observation, it can be concluded that the luteotropin of the pig is controlled by an inhibitory influence of the central nervous system.

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APPENDIX

Table 5. Survival of pigs after adenohypophysial autotransplantation hypophysectomy

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs: Survival period (days)	Disposition of pigs: Condition causing death
2392	H	99	31.8	1	Muscular weakness and aphasia
2470	A	92	29.1	5	Muscular weakness and aphasia
2461		95	29.5	0	Stress at hypophysectomy
2990	H	99	25.4	23	Electrolyte imbalance
2971	H	100	27.3	1	Muscular weakness and aphasia
2915	H	97	25.9	4	Muscular weakness and aphasia
2901	A	97	27.7	2	Muscular weakness and aphasia
2943	A	100	25.4	89	Electrolyte imbalance
2995	A	101	27.7	12	Electrolyte imbalance
2794	A	97	27.3	1	Muscular weakness and aphasia
4064	A	100	32.7	78	Stress of laparotomy

[a] H = hypophysectomy, and
A = adenohypophysial autotransplantation.

Table 5. (Continued)

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs	
				Survival period (days)	Condition causing death
4206	H	103	34.5	29	Electrolyte imbalance
4207		103	33.6	0	Stress at hypophysectomy
4221	A	101	35.4	4	Muscular weakness and aphasia
4223	H	101	33.6	73	Electrolyte imbalance
4225	A	108	38.2	67	Electrolyte imbalance
4302		107	38.6	0	Stress at hypophysectomy
4310	H	107	34.1	6	Electrolyte imbalance
4312	A	110	35.4	40	Electrolyte imbalance
4314	A	110	35.4	44	Electrolyte imbalance
4340		107	26.4	0	Stress at hypophysectomy
4345		107	28.6	0	Stress at hypophysectomy
4343	H	111	31.4	77	Stress of laparotomy
4410		109	34.1	0	Stress at hypophysectomy

Table 5. (Continued)

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs: Survival period (days)	Disposition of pigs: Condition causing death
4490	A	112	38.6	61	Electrolyte imbalance
4563	A	104	40.0	67	Killed - no response to gonadotropins
4564	A	111	38.2	51	Electrolyte imbalance
4643	A	133	63.2	122	Killed - no response to gonadotropins
4653	A	134	69.1	127	Completed experiment
4652	A	134	76.8	107	Electrolyte imbalance
4690	A	140	65.0	28	Electrolyte imbalance
4691	A	134	63.6	19	Electrolyte imbalance
4692	A	140	65.0	64	Completed experiment
4694	A	147	65.9	6	Electrolyte imbalance
5700	A	100	40.0	78	Completed experiment
5701	A	102	42.3	22	Electrolyte imbalance

Table 5. (Continued)

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs	
				Survival period (days)	Condition causing death
9021	A	97	25.0	43	Electrolyte imbalance
9037		104	25.0	0	Stress at hypophysectomy
9090	A	102	29.5	64	Completed experiment
9134	A	105	28.6	63	Electrolyte imbalance
9300	A	106	27.3	85	Electrolyte imbalance
9301	A	104	33.6	86	Electrolyte imbalance
9312	A	105	35.4	64	Completed experiment
9333		105	34.1	0	Stress at hypophysectomy
9355	A	104	35.4	64	Completed experiment
9361	A	101	37.3	112	Completed experiment
9370	A	104	32.3	73	Electrolyte imbalance
9411	A	123	37.3	1	Muscular weakness and aphasia
9422	A	125	40.9	7	Electrolyte imbalance

Table 5. (Continued)

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs	
				Survival period (days)	Condition causing death
9433	A	145	61.4	50	Electrolyte imbalance
9440		106	35.0	0	Stress at hypophysectomy
9736	A	109	33.6	64	Completed experiment
9740	A	110	32.3	16	Electrolyte imbalance
9921	A	113	28.2	20	Electrolyte imbalance
9963		114	26.8	0	Stress at hypophysectomy
6063	H	111	30.0	71	Completed experiment
6040	A	117	31.8	83	Electrolyte imbalance
6111	H	115	32.7	24	Electrolyte imbalance
6110	A	120	39.5	95	Completed experiment
6210	H	113	27.7	17	Electrolyte imbalance
6231	H	115	29.5	4	Muscular weakness and aphasia
6402	H	115	28.2	1	Muscular weakness and aphasia

Table 5. (Continued)

Pig no.	Treatment[a]	Age at hypophysectomy (days)	Weight at hypophysectomy (kg)	Disposition of pigs	
				Survival period (days)	Condition causing death
6404	H	103	24.5	70	Electrolyte imbalance
6502	H	104	29.5	69	Completed experiment
6550	H	103	27.3	3	Muscular weakness and aphasia
6624	H	100	29.5	2	Muscular weakness and aphasia
6701	H	105	34.1	1	Muscular weakness and aphasia
6720	H	104	34.1	2	Muscular weakness and aphasia
6723	H	109	32.7	140	Living - no response to gonadotropins
7006	H	104	31.4	1	Muscular weakness and aphasia
7000	H	113	29.1	2	Muscular weakness and aphasia
7033	H	106	27.3	3	Muscular weakness and aphasia